

# **Technical Report**

**NREL Subcontract #HAW -3-11181-06**

## **Evaluation of Alternate Pretreatment and Biomass Fractionation Processes-The Ammonia Fiber Explosion Process (AFEX)**

by

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# Table of Contents

	Page
<b>Introduction.....</b>	<b>1</b>
Milling has both advantages and disadvantages.....	1
Early Observation Reveal Differences Between Final Hybrid Poplar.....	1
Unfortunate Milling of All the Final Substrate to Below 40 Mesh.....	2
 <b>Pretreatment Optimization Review.....</b>	 <b>3</b>
Experimental Plan Review.....	4
Explanation of the Original Plan Parameters.....	4
Hybrid Poplar Description for 1-14-94 Observations.....	6
The New Design is Formulated With Respect to Each Previous Set of Observations.....	8
Substitute Hybrid Poplar Particle Size Description and Inventory.....	9
Observations Based on Substitute Hybrid Poplar.....	12
Temperature Threshold is Set By Previous Observations.....	13
Optimum Water Load is Set.....	13
Equilibration of Ammonia With the Biomass.....	14
Reduction in the Initial Hydrolysis Time for Faster Optimization.....	15
Data Set Review and Comments.....	16
 <b>Summary.....</b>	 <b>20</b>
Substrate Composition and Sieving/Milling Techniques Explain Variations in Results.....	20
Review and Commentary on Ethanol Experimental Data Sets.....	20

## Tables

Table 1a. The Original Experimental Plan AFEX Parameters.....	4
Table 1b. The Original Experimental Plan Enzyme and Solids Loads.....	5
Table 1c. The Original Experimental Plan Glucose Digestibility Results.....	5
Table 1d. Original Experimental Plan Results in % Glucan Digestibility.....	5
Table 2 The Enzyme and Solid-loading on Best AFEX Original Observations.....	6
Table 3a. The Best Results from the Original Experimental Plan AFEX Parameters.....	6
Table 3b. The Best Results from the Original Experimental Plan AFEX Parameters.....	7
Table 4. Substitute NREL Hybrid Poplar Sample List.....	8
Table 5. NREL Final Substrate Compositional Analyses-Final Results.....	9
Table 6. NREL Actual Subcontract Hybrid Poplar.....	10
Table 7. Test Sample Analyses of Alpha Cellulose.....	11
Table 8. NREL Test Sample Analyses Final Results.....	12
Table 9. AFEX High/Low Temperature 1X, 2X and 3X NH <sub>3</sub> Observation.....	14
Table 10. AFEX Extended Time (Water held constant) 2X Vs. 3X NH <sub>3</sub> Observations.....	16
Table 11. AFEX Optimized Temperature Wet Vs. Dry 1X Vs. 2X NH <sub>3</sub> Observations.....	17
Table 12. AFEX Optimized Temperatures, Larger Particles, Wet Vs. Dry Observations.....	17
Table 13. AFEX Optimized Temperature, Reduced Ammonia Wet Vs. Dry, Time Increase.....	18
Table 14. AFEX Observations on MBI Milled Poplar to Reproduce Previous Results.....	18
Table 15. AFEX Observations on MBI Milled Poplar.....	18

## Acronyms and Abbreviations

AFEX	Ammonia Fiber Explosion
AQUAFEX	Ra Energy Pretreatment Technology
DOE	Department of Energy
GC	Gas chromatograph
MBI	Michigan Biotechnology Institute
NREL	National Renewable Energy Laboratory
QA/QC	Quality assurance/quality control
YSI	Yellow Springs Instrument

## Appendices

Energy and Mass Balance Summary.....Appendix A

Total Calculations and Energy and Mass Balance Report.....Appendix B

SSF (Simultaneous Saccharification and Fermentation) Data Report.....Appendix C

# Introduction

The investigation of Ammonia Fiber Explosion (AFEX) pre-treatment on hybrid poplar for the production of fuels and chemicals by fermentation (post enzymatic hydrolysis) is reviewed in the following report. The current explanation of the effects of the AFEX treatment is as follows: The lignocellulosic biomass fibers are soaked to saturation with a predetermined amount of liquid ammonia under a pressure of about 180 psia to about 400 psia at temperatures between 30 and 90°C. Several effects are taking place during this soaking phase. The ammonia absorbs onto the biomass and decrystallizes the cellulose and helps to solubilize the hemicellulosic fraction of the biomass. The absorption of ammonia and the decrystallization are taking place while ammonia is coming to equilibrium throughout the biomass and total volume of the reactor. After a thorough ammonia soak the pressure is instantaneously released causing an explosion through a ball valve which vents the ammonia fumes to a blowdown tank at least 50 times the volume of the reactor. It is speculated that hydrogen atoms are ripped from the biomass causing a form of decrepitation to the crystalline linkages.

The original experimental plan was written based upon the four parameters which did not include particle size. The parameters referred to are water load, ammonia load, reactor residence time and temperature. National Renewable Energy Laboratory (NREL) provided eight hybrid poplar samples which actually consisted of only three different particle sizes. Approximately 3 kilograms were -60 mesh, 1.0 kilogram was at -60 mesh to + 80 mesh and 1.7 kilograms was -1/16 inch to + 60 mesh. These were provided with the hope that we could determine the best particle size for all observations. A list can be found in Table 4. Once it was determined at what particle size the pretreatment would best be optimized, the milling to the selected particle size would be done. Several observations were made using short hydrolysis durations to determine best particle size. Glucose yields from these observations are listed in Tables 1c, 1d and 3a. The data in Table 3a of Set B on page 6 is based on biomass with water pre-equilibrated for longer than one hour before ammonia was loaded and residence time of 30 minutes plus a 25 minute ramp time (a total exposure time of 55 minutes).

## MILLING HAS BOTH ADVANTAGES AND DISADVANTAGES

Milling the biomass before the ammonia treatment can help in producing higher glucose and xylose yields at higher rates of enzymatic reaction than could be achieved at a particle size as large as 2mm. If the surface area is excessive, the ammonia saturation into the biomass is too complete and even after the explosion (which drives off 84% of the total ammonia Latimer 1992 unpublished). The excess ammonia causes a high pH directly on the biomass fibers even after a two hour soak in 4.8 pH buffer. The high pH on the biomass will inhibit the reactivity of the cellulase and beta glucosidase enzymes in the hydrolysis reaction since enzyme conformation (directly related to hydrolysis efficiency and rate) is critically dependent upon substrate surface pH. The "easily saturated" aspect of finely ground (40 mesh and below) AFEX material can be used to help reduce the amount of ammonia necessary to induce an effective treatment through particle size reduction and reducing the effective load of ammonia to about one-half the amount which would be necessary at a larger particle size (such as 2mm).

## **EARLY OBSERVATIONS REVEAL DIFFERENCES BETWEEN FINAL SUBSTRATE, MILLED HYBRID POPLAR, AND THE SUBSTITUTE MILLED HYBRID POPLAR SUBSTRATE**

It should also be noted that the particle size of -60 mesh as compared with the -1/16 inch to + 60 mesh did seem to exhibit more resistance to enzyme susceptibility, even when AFEX treated, due to the compacting effect of the "Winona" milling done at Michigan Biotechnology Institute (MBI). Note that the untreated hybrid poplar sent back from MBI used in both data Sets 6 and 7 in Tables 14 and 15 rendered significantly lower glucan digestibility at the -60 mesh particle size than did the NREL - 60 mesh untreated hybrid poplar from the NREL sieved substitute samples. This substitute hybrid poplar was presented (pre-ground and pre-sieved) to Ra Energy Ltd. for preliminary AFEX observations to determine the particle size reduction necessary to complete the pretreatment observations. It is speculated that knife-milled hybrid poplar (the pre-ground substitute poplar sent from NREL) has a better response to enzymes than "winona" milled poplar even as an untreated substrate at -60 mesh. See the untreated hybrid poplar glucan digestibility results for NREL milled and sieved wood on Sets 1, 2, 3, and 4 in Tables 9,10,11 and 12 on pages 14, 15 and 16. The MBI milled wood hydrolysis results for the untreated samples are in Sets 5, 6 and 7 in Tables 13, 14 and 15 on pages 18 and 19.

## **UNFORTUNATE MILLING OF ALL THE FINAL SUBSTRATE TO BELOW 40 MESH**

It is fortunate that a substitute experimental hybrid poplar was sent to Ra Energy Ltd. by Daniel Hsu early in the course of this subcontract. This material was used for several AFEX pretreatment observations prior to running the actual samples which were run using the particle sizes as milled by NREL. Even the analytical evaluation of compositional profiles appeared to be very different between the original batches of hybrid poplar test sample sent from NREL (these chemical analyses are logged in Table 8) and the compositional hybrid poplar analyses data for hybrid poplar milled at MBI (recorded in Tables 5 and 6). Observations from data Set 4 were run on -1/16 inch to + 60 mesh hybrid poplar particle size. AFEX sample Set 4 had the highest total "Glucan Digestibility" of all the hybrid poplar digestibility yields. Unfortunately most other experimental observations were done on - 60 mesh particle size.

Observations from Sets 6 and 7 at -40 mesh have some rerun hydrolyses data on MBI milled hybrid poplar. Due to lower glucose yields in these experimental results on the -60 mesh hybrid poplar test sample and a matching low alpha cellulose standard glucose yield, these sets were both run twice. The first run on each of these sets utilized the NREL enzymes which were supplied in the beginning of the project. The second runs utilized a new supply of NREL enzymes. The second run for these sets are in parentheses in Tables 14 and 15 on pages 18 and 19.

# Pretreatment Optimization Review

It can be demonstrated in the following review of experimental parameters and resulting data sets that various combinations of AFEX settings exist which can show optimum glucan digestibility for the parameters listed :

ammonia concentration to biomass
water concentration to biomass
equilibration time
reactor temperature
particle size (surface area to biomass ratio)

Combinations of treatment parameters exist which can be applied to a large number of commercial situations at large to achieve excellent pretreatment. The concept of easy ammonia recovery and recycle as demonstrated in this subcontract greatly enhances the economic and scientific feasibility of this technology. One of several aspects which was not investigated due to time constraints is the possibility of multiple explosions which is a patented aspect of AFEX technologies. This multiple explosion concept can be executed using a technique called "Aquafex". This subcontract was executed as a preliminary study of AFEX pretreatment of hybrid poplar and should be considered as such. It is the belief of Ra Energy Limited's technical and research management that with time and experimentation the ideal pretreatment conditions for hybrid poplar can be found using the existing AFEX technologies. This is only the first phase of investigation and only glucan digestibility is reviewed.

## EXPERIMENTAL PLAN REVIEW

This report contains a review of the experimental data generated in the NREL Subcontract #HAW-3-11181-06 with Ra Energy Limited. First it must be explained that the original experimental plan parameters as set forth in Table 1 of the originally submitted experimental plan were followed to some degree. Time constraints, non-uniform ramp times (which were a result of setting parameters for temperature, moisture and ammonia without any respect to time) and the desire to achieve worthwhile data, dictated that the experimental parameters be altered as set forth in data Sets 1-7 in tables 9-15 and as recorded on pages 9-15. The respective graphics and charts are listed in the figures and tables section of the table of contents. A copy of the original experimental plan parameters in Table 1 can be found on page 3 of this text.



## Original Experimental Plan AFEXING Parameters

Table 1a. The Original Experimental Plan AFEX Parameters

<u>Run#</u>	<u>Time(min.)</u>	<u>Temp(C)</u>	<u>NH<sub>3</sub>(kg/kg biomass)</u>	<u>Moisture (kg/kg biomass)</u>
1	30	90	2.0	0.375
2	30	30	2.0	0.375
3	10	90	2.0	0.375
4	10	30	2.0	0.375
5	20	60	3.0	0.6
6	20	60	3.0	0.15
7	20	60	1.0	0.6
8	20	60	1.0	0.15
9	20	60	2.0	0.375
10	30	60	2.0	0.6
11	30	60	2.0	0.15
12	10	60	2.0	0.6
13	10	60	2.0	0.15
14	20	90	3.0	0.375
15	20	90	1.0	0.375
16	20	30	3.0	0.375
17	20	30	1.0	0.375
18	20	60	2.0	0.375
19	30	60	3.0	0.375
20	30	60	1.0	0.375
21	10	60	3.0	0.375
22	10	60	1.0	0.375
23	20	90	2.0	0.6
24	20	90	2.0	0.15
25	20	30	2.0	0.6
26	20	30	2.0	0.15
27	20	60	2.0	0.375

The initial observations based on the original experimental plan all yielded 35% or less of the 50% glucose available in the hybrid poplar substitute substrate. (See Table 1a for parameters, Table 1 b for enzyme and solid loads and Table 1c for original results.) Contained within Table 4 are the results of an AFEX run which appears to have the best results at the parameters which are listed under the table:

### EXPLANATION OF THE ORIGINAL PLAN PARAMETERS

The original experimental plan design includes time exposures in the AFEX reactor of less than 30 minutes (as written per consultants Dr. Bruce Dale & Dr. Larry Douglas). Several of the initial observations using 30 minutes and less of exposure time with various moisture and ammonia loads revealed that very little pretreatment was being achieved especially when water was loaded just prior to the AFEX treatment (see Tables 1a, 1b, 1c, 1d, 2, and 3). Prior work on other less recalcitrant substrates (i.e. Coastal Bermuda grass) revealed that time exposures of less than thirty minutes were not successful in converting any significant portion (greater than 30% of the total biomass) of the biomass into a glucose yielding substrate by using any moisture or ammonia load unless a presoak (equilibration time) for water and or ammonia of at least thirty minutes was implemented. (Latimer 1992 unpublished).

**Table 1b. The Original Experimental Plan Enzyme And Solid Loads**

Hybrid Poplar Sample DESCRIPTION for the 1-4-94 Observation-60 to + 80 mesh

First set of Preliminary observations-Set A

<u>Sample</u>	<u>% Solids</u>	<u>Cellulase</u>	<u>Cellobiase</u>	<u>Initial pH</u>
A	5%	5IU	20IU	4.8
B	10%	5IU	20IU	4.8
C	5%	10IU	20IU	4.8
D	10%	10IU	20IU	4.8
E*	5%	5IU	20IU	4.80
F	10%	10IU	20IU	4.8

\*Samples A & B are untreated. Samples C through F are AFEXED at the parameters listed :

AFEX settings were thirty minutes at 90°C, 2 kg NH<sub>3</sub>/kg poplar and 0.3kg H<sub>2</sub>O/kg poplar. All samples are size -60 to + 80 mesh.

**Table 1c. The Original Experimental Plan Glucose Digestibility Results**

Set A All samples are size -60 to + 80 mesh.

Actual YSI Glucose Analyzer readings in grams per liter

<u>Glucose 24 hour</u>	<u>Glucose 48 hour</u>	<u>Glucose 72 hour</u>
	<u>pH</u>	
A =2.62	A = 1.43 4.7	A = 1.50
B = 5.64	B = 9.90 4.5	B = 4.64
C = 4.45	C = 4.95 4.8	C = 3.52
D = 8.26	D = 8.67 4.6	D = 10.3
E = 7.01	E = 7.4 4.8	E = 8.08
F=12.6	F=13.3 4.8	F=13.20

**Table 1d. Original Experimental Plan Results in % Glucan Digestibility**

Set A All samples are size -60 to + 80 mesh.

<u>Glucose 24 hour</u>	<u>Glucose 48 hour</u>	<u>Glucose 72 hour</u>
A =2.5%	A =<2.5%	A =<2.5%
B =2.7.%	B =<2.7%	B =<2.7%
C =4.27%	C = 4.75%	C =<4.75%
D =3.96%	D = 4.16%	D =<4.16%
E = 6.72%	E = 7.10%	E = 7.75%
F = 6.05	F = 6.38%	F = <6.38%

The results in this table represent yields from hybrid poplar samples which were not pre-soaked before AFEX pretreatment was applied. Note that none of the glucan digestibility's exceed 8%.

## HYBRID POPLAR DESCRIPTION FOR 1-14-94 OBSERVATIONS-1/16" TO + 60 MESH

AFEX treated on 1-10-94

A 100gram sample soaked in 0.3kilogram of water per kilogram of substrate for 30 minutes prior to being loaded into the AFEX reactor with 300grams of Ammonia (3:1 NH<sub>3</sub> to biomass). This mixture was taken to 90°C (194°F) and held there for thirty minutes. Total ammonia exposure time is equal to 56 minutes.

**Table 2. The Enzyme and Solid-loading on Best AFEX Original Observations**

<u>Sample</u>	<u>% Solids</u>	<u>Cellulase</u>	<u>Cellobiase</u>	<u>Initial pH</u>
A	5%	5IU	20IU	5.05
B	10%	5IU	20IU	5.05
C	5%	10IU	20IU	5.00
D	10%	10IU	20IU	5.05
E*	4.67%	5IU	20IU	5.00

\*Sample A through D are AFEXED at the above parameters Sample E is untreated. All samples are size 1/16" to + 60 mesh.

Sample Description: NREL original observational substitute hybrid poplar which was milled by NREL subcontractor to a particle size of (-1/16" to + 60 mesh)

**Table 3a. The Best Results from the Original Experimental Plan AFEX Parameters.**

**Preliminary Experiment Set 1** particle size of (-1/16" to + 60 mesh)

<u>Glucose 24 hour</u>	<u>Glucose 48 hour</u>	<u>Glucose 72 hour</u>
	<u>pH</u>	
A = 3.59	A = 4.99 4.7	A = 5.36
B = 8.17	B = 9.90 4.5	B = 10.9
C = 6.77	C = 6.27 4.8	C = 8.40
D = 11.4	D = 15.2 4.6	D = 15.6
E = 3.04	E = 3.46 4.8	E = 3.46

**% Glucan Digestibility Set 1** particle size of (-1/16" to + 60 mesh)

<u>Glucose 24 hour</u>	<u>Glucose 48 hour</u>	<u>Glucose 72 hour</u>
	<u>pH</u>	
A = 14.9%	A = 20.79% 4.7	A = 22.33%
B = 17.02%	B = 20.63% 4.5	B = 22.7%
C = 28.21%	C = 26.12% 4.8	C = 35.00%
D = 23.75%	D = 31.66% 4.6	D = 32.5%
E = 12.66%	E = 14.41% 4.8	E = 14.42%

It is unfortunate that the -1/16 inch to + 60 mesh particle was not the size milled at MBI. The best results appear to have been achieved at this particle size in both the treated and the untreated samples.

**Table 3b. The Best Results from the Original Experimental Plan AFEX Parameters.**

**Preliminary Experiment Set 2** particle size of (-60 mesh to + 80 mesh)

<u>Glucose 24 hour</u>	<u>Glucose 48 hour</u>	<u>Glucose 72 hour</u>
24 Hours	48 Hours	72 Hours
A =3.23	A =4.83	A =4.89
B =3.40	B =6.51	B =9.22
D =11.3	D =12.6	D =11.7
E =2.97	E =3.10	E =3.41

Sample C was contaminated.

**% Glucan Digestibility Set 2** particle size of (-60 mesh to + 80 mesh)

<u>Glucose 24 hour</u>	<u>Glucose 48 hour</u>	<u>Glucose 72 hour</u>
24 Hours	48 Hours	72 Hours
A =13.46%	A =20.13%	A =20.38%
B = 7.08%	B =13.56%	B =19.21%
D =23.54%	D =26.25%	D =24.38%
E =12.37%	E =12.92%	E =14.21%

Sample C was contaminated.

To calculate the % of glucose yielded use 50gms/lit for the 100gms/lit used in the 10% solid samples. Divide the Glucose yield by 50gms/lit (for the 5% solids loaded) or by 100gms/lit (for the 10% solids samples) to give the final percentage of glucose yielded multiply the answer by 0.48 which will equal the glucan digestibility which is listed for these preliminary observations in the above sets of glucan digestibility. Total theoretical glucose percent in the hybrid poplar is 48 % according to the analytical test data in Tables 5,6,7 and 8.

### ***The New Design Was Formulated With Respect to Each Previous Set of Observations***

The specific experimental results listed in Tables were obtained through several consecutive sets of observations (a total of seven sets of observations were formulated in this manner) which were structured using the data generated from each previously numbered set of observations. Several initial observations (the first data set which is Set 1) using thirty minute time exposures and various moisture and temperature variables revealed that very little pretreatment was being achieved using thirty minute exposures without presoaking the hybrid poplar samples in the respective water load for at least one hour before AFEX treatments were applied. Since the original experimental plan (statistically generated by Dr., Larry Douglas, formerly of SERI, using parameters suggested by Dr. Bruce Dale, Currently of Texas A & M University) utilized no time period greater than 30 minutes in total (some as short as ten minutes) the original experimental plan had to be investigated and redesigned. The samples utilizing water loading with the original time spans for treatment, yielding sometimes 10 to 15% of the glucose with no greater than 20% of the theoretical glucose through enzyme hydrolysis in samples with reactor residences times of 10 and twenty minutes.

### ***Time is a Critical Variable of The AFEX Water Loading Concept***

It is known among experienced AFEX users, of which there are very few, that to see any improvement in the AFEX process due to water loading, the water must be added and the biomass soaked for at least thirty minutes in advance of the ammonia exposure. This parameter was not included in the original experimental plan or the edited plan. The proposed 30 minute reaction time exposures must also include a ramp time which is lengthened with each degree increase in the final temperature of exposure. This ramp time is a parameter which is dictated by the current reactor design which cannot be avoided and should not be ignored. No method to account for the moisture equilibration time was being employed.

It is Ra Energy Limited's belief that the best commercial parameters for ammonia recovery would utilize no added moisture loading at all (utilizing the natural moisture content). It was decided that in order to compare temperature without interference from variations in ammonia exposure duration, (Latimer/Ra Energy Ltd.) that all durations (soak or equilibration time for biomass in the ammonia), regardless of temperature would be made equal by extending all temperature exposure durations to a preset number of minutes greater than thirty minutes. During the course of executing the AFEX parameters which held time constant for all moisture and temperature and ammonia variations the best settings for all parameters for glucan digestibility and glucose yield became apparent.

**Table 4. Substitute NREL Hybrid Poplar Sample List.**

	Sample #	Weight in kgs	Particle Size
	1	0.7733	- 60 mesh
	2	0.7179	- 60 mesh
	3	0.4989	- 60 mesh+ 80 mesh
	4	0.8044	-1/16"+ 60 mesh
	5	0.7974	- 60 mesh
	6	0.4805	- 60 mesh+ 80 mesh
	7	0.695	- 60 mesh
	8	0.906	-1/16"+ 60 mesh

### Substitute Hybrid Poplar Particle Size Description and Inventory

A list of a substitute hybrid poplar sent to Ra Energy laboratories is listed in Table 4 on page 10. These samples were provided for initial observations and subsequent determination of the particle size needed for delivery of the subcontract specified hybrid poplar. A compositional analysis of this substrate is included below in Table 5.

**Table 5. NREL Final Substrate Compositional Analyses - Final Results.**

#### Hybrid Poplar Test Sample Analytical Results

##### CHEMICAL ANALYSIS:

**SAMPLE:** NREL Actual Wood Substrate for Final Pretreatment Analysis (milled at MBI by Ra Energy Ltd.)  
Particle Size: - 60 mesh

**DATE:** May, 1994

	@105°C	@45°C
% Total Solids	98.01	99.88
% Moisture	1.99	0.12
%Glucose		(41.25)
%Glucan (glucose x 0.90)		37.13
%Xylose		(18.27)
%Xylan (xylose x 0.88)		16.08
%Klason Lignin		25.85
%Acid Soluble Lignin		2.19
%Ash (dry wt. basis)		2.06 (average of -60 & -80 mesh)
		-----

#### Glucose and xylose recovery after two stage sulfuric acid hydrolysis:

% Glucose recovered:	96.80
% Xylose recovered	90.41

**Table 6. NREL- Actual Subcontract Hybrid Poplar**

**CHEMICAL ANALYSIS:**

**SAMPLE:** NREL Actual Wood Substrate for Final Pretreatment Analysis (milled at MBI by Ra Energy Ltd.)  
Particle Size: - 40 mesh

**DATE:** May, 1994

	@105°C	@45°C
% Total Solids	97.30	untested
% Moisture	2.7	untested
%Glucose		untested
%Glucan (glucose x 0.90)		
%Xylose		untested
%Xylan (xylose x 0.88)		
%Klason Lignin	25.77	
%Acid Soluble Lignin	2.75	
%Ash (dry wt. basis)	1.43	
	-----	

**NREL glucose standard:** 1.02mg/ml (HPLC); 1.01 mg/ml(YSI)

**NREL ethanol standard:** (QA-ETOH-08): 31.26 mg/ml or **3.96 %** (v/v) (GC)

**Table 7. Test Sample Analyses of Alpha Cellulose**

**CHEMICAL ANALYSIS:**

**SAMPLE:** alpha - Cellulose (NREL)

**DATE:** May, 1994

	<b>@105°C</b>	<b>@45°C</b>
<b>% Total Solids</b>	95.75	97.85
<b>% Moisture</b>	4.25	2.15
<b>%Glucose</b>		
<b>%Glucan</b> (glucose x 0.90)		99.95
<b>%Xylose</b>		
<b>%Xylan</b> (xylose x 0.88)		
<b>%Klason Lignin</b>		
<b>%Acid Soluble Lignin</b>		
<b>%Ash (dry wt. basis)</b>		0.24
		-----

**Glucose and xylose recovery after two stage sulfuric acid hydrolysis:**

<b>% Glucose recovered:</b>	<b>96.80</b>
<b>% Xylose recovered</b>	<b>90.41</b>



**Table 8. NREL Test Sample Analyses Final Results.**

**CHEMICAL ANALYSIS:**

**SAMPLE:** (QA-W0-08), NREL Wood Standard

**DATE:** June 17, 1994

	@105°C	@45°C
% Total Solids	95.40	96.32
% Moisture	4.60	3.68
%Glucose		(48.67)
%Glucan (glucose x 0.90)		43.80
%Xylose		(17.80)
%Xylan (xylose x 0.88)		15.66
%Klason Lignin		25.85
%Acid Soluble Lignin		2.19
%Ash (dry wt. basis)		1.25
		-----

NREL glucose standard: 1.02mg/ml (HPLC); 1.01 mg/ml(YSI)

NREL ethanol standard: (QA-ETOH-08): 31.26 mg/ml or 3.96 % (v/v) (GC)

**OBSERVATIONS BASED ON SUBSTITUTE HYBRID POPLAR**

The initial experimental plan was formulated based on using a steam generator to bring samples to a set exposure temperature without enduring the ramp time normally encountered in the AFEX reactor design. The steam generator concept, although excellent in theory, posed many practical problems for so small a reactor as the current AFEX reactor design. The steam generator was aborted due to time and technical constraints. Some AFEX work was done using a microwave to heat the treatment water for some preliminary observations. It was apparent that water loading with water preset to the predetermined observation temperature still required an equilibration time to show a treatment effect.

It was also decided that due to the new experimental design that the amount of water present (and the length of soak time) in the reaction was only important because of the affinity that ammonia has to water. This extreme affinity induces a faster rate of bringing the ammonia (as long as the minimum concentration of ammonia necessary for a good pretreatment was present) into equilibrium with the biomass. Temperature should have played a role in the rate of this equilibration, but temperature has proved to be not as significant as longer time exposure in this and previous AFEX work done in a set volume reactor (Latimer 1992 to present unpublished) for optimizing the penetration of ammonia into the biomass.

The goal of this work was to isolate temperature, total reactor residence time and water loading effect to such a degree as to isolate the ideal setting to run this biomass into a reactor under a commercial setting.

### ***Temperature Threshold Set By Previous Biomass Deterioration Observed Above 90°C***

The first threshold that was determined was the maximum temperature threshold. Early observations on the hybrid poplar in Set 1 Table 9 reveal the results of the chosen threshold around 90°C. This threshold is defined by a glucan digestibility drop as an indicator of heat damage to the biomass. The temperature at which this damage occurs is a few degrees below 100° C and the result is an apparent reduction in available glucose from the pretreated hybrid poplar. The 90°C maximum was set in previous AFEX treatment observations using sugar cane bagasse. Bagasse has a similar high lignin content and a protein content of less than 1% just as hybrid poplar. The choice not to exceed 90°C was based upon the commercial goal of using the minimum energy input necessary to achieve a good AFEX treatment and staying comfortably below the biomass damage threshold. The high temperature chosen was 90°C and the low temperature was 30°C. Experimental parameters were chosen between these two extremes for definitive digestibility results. The first set of parameters used the highest and lowest temperatures and the highest ammonia concentration of 3kg/kg dry biomass and the lowest ammonia concentration of 1kg/kg of dry biomass. Set 1 Table 9 Observation 5 was run in the ammonia concentration mid range of 1.5kg/kg of dry biomass and looked identical in glucan digestibility to the high temperature (90°C) 1kg ammonia to 1kg dry biomass of Observation 3 of the same set. Since observation 5 has 0.5kg of water /kg biomass and 1.5kg of ammonia /kg biomass the pressure at 30°C had an effect similar to the digestibility observed in Observation 3, where the ammonia load is 1kg of ammonia /kg biomass and no water at 90°C. Both of these observations resulted in near equal overall pressure. The experiment Set 1 has 5 observations at totally different settings, demonstrating the importance of the interplay in combinations of factors to produce two overall effects 1) pressure and 2) ammonia diffusion rate. The current "set volume" system results in pressure being dependent upon temperature and the vapor pressures produced from interdependent combinations of temperature, ammonia and water concentrations. The equilibrium of water and ammonia with the biomass and the diffusion intensity of the ammonia into the biomass over a set time period appear to be dependent upon any moisture which may be naturally equilibrated within the biomass before AFEX treatment, added moisture while pretreating with ammonia and pressure/temperature. The final outcome of any one AFEX treatment although dependent upon all factors in the experimental design can be comparatively identical in glucan digestibility with drastically different ammonia and energy inputs.

### ***Optimum Water Load is Set Based on Prior AFEX Results on Multiple Substrates***

The fact prevailed throughout all of the work on multiple substrates exposed to AFEX treatments that those samples with 0.5 kilograms of water per kilogram of biomass (if run at the optimum temperature between 70 to 85°C for most every substrates) yielded the greatest glucan digestibility when compared to samples run at any other moisture content in the current 4 liter reactor volume. When water loading exceeds 0.5 kilogram per kilogram of biomass there is an obvious diluting effect on the ammonia and any subsequent final ammonia effect. When water loading is lower than 0.5 kilograms per kilogram of dry biomass there appears to be less ammonia absorption and dispersion throughout the biomass and therefore a reduction in the pretreatment intensity which results in a lower glucose yield.

This result is due to the high affinity of ammonia to water which should be already equally distributed throughout the biomass by the time the ammonia is loaded. The high affinity of ammonia to water proves very useful in extracting ammonia from the biomass rendering the biomass about 99% ammonia free after washing, less than 1% is left on the biomass (Latimer 1992 unpublished contract work done under Dr. Bruce Dale for Earnest Stuart of AFEX Corporation, lab books exist to verify this fact).

The first set of parameters used for AFEX treatment explored 30 and 90°C temperatures both dry and with 0.5kg of water/kg of dry hybrid poplar wood. We did not explore 30°C degrees for any time period greater than 35 minutes simply because of the low glucose reading obtained at this temperature. It should

be noted that hybrid poplar at 30°C (Set 1, Observation 5 in data Table 9) at 1.5:1 ammonia to biomass yielded a 10% increase over glucose yield at 30°C 1kg of ammonia to 1kg of biomass for a six minute shorter time duration when water (which required a presoak of at least an hour) was compared to dry biomass with a 1:1 ammonia to biomass ratio (Set 1 Observation 1).

### ***Equilibration of Ammonia With the Biomass is Based on Water Load and Soak Time***

The problem which occurred in trying to compare these dry vs. 0.5 kilogram of water per kilogram of dry wood observations was the unrecorded water soak time of one hour on the biomass. If Observation 1 which is a dry biomass was given equal time (one hour) for the ammonia to soak (equilibrate) into the biomass as was allowed for water (and consequently the ammonia which, due to its extremely high affinity to the moisture readily absorbs into the core of the fibers of the already soaked wood sample) treatment would appear more uniform for both wet and dry observations. This concept can be appreciated when reviewing data in Tables 1b, which shows solids concentrations and enzyme loading, Table 1c, which shows actual glucose readings and Table 1d, which lists glucan digestibility in the unsoaked water AFEX treated samples.

Pretreating the poplar samples with water and allowing a period of soaking time before ammonia addition is not a problem as long as the water does not contaminate the ammonia which we propose to recycle. Since this has yet to be determined we continue the investigation assuming there are at least two possibilities: (1) that some water escapes with the ammonia upon explosion (worst case would be that the total mass of water is bound and released with the ammonia. (2) the second assumption is that thermodynamically it is not possible for the water to be driven from the sample as rapidly as the ammonia and therefore most or possibly all of the water is left behind due to the temperature drop which occurs as the ammonia changes from its compressed liquid state in the micro-crevices of the ground and soaked biomass.

The second assumption becomes more feasible when the treatment is conducted at the lowest possible reactor temperature that yields closest to the optimum result. Continuing along these thoughts the experimental design was tailored to determine the best lowest temperature as well as the best dry treatment possible as set out in data sets 3, 5, 7 and 8.

**Table 9. AFEX High/Low Temperature, 1X, 2X and 3X NH<sub>3</sub> Observations**

### **Enzyme Saccharification of Hybrid Poplar Test Samples (NREL Protocol #009):**

**SET # 1** ( Particle Size: - 60 mesh)-NREL knife milled and sieved to particle size specified.

<b>AFEX Treatment Conditions:</b>		<b>DIGESTIBILITY:</b>
		<b><u>72hrs</u></b>
01-	30° C, 1:1 NH <sub>3</sub> , dry (35 min.)	21.1 %
02-	30° C, 3:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood (29 min.)	25.1%
03-	90° C, 1:1 NH <sub>3</sub> , dry (53 min.)	30.2 %
04-	90° C, 3:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(56 min.)	46.3 %
05-	30° C, 1.5:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(29 min.)	30.1 %
06-	Untreated hybrid poplar	15.0 %

### ***Reduction in The Initial Hydrolysis Time is Necessary to Accomplish the Needed Number of Observations to Determine Optimum Particle Size***

This original set of hydrolysis data was run only until the seventy second hour for purposes of determining the best observation rapidly and preparing a new set of AFEX parameters for the second set of observations. Both Set 1 and Set 2 were run without an alpha cellulose standard due to the fact that we were using these observations solely for purposes of exacting a new experimental plan as well as determining a target particle size for pretreatment, hydrolysis and fermentation. Set 1, Observations 2 and 4 as well as Set 1, Observations 1 and 3 were used to observe the results of the extremes of temperature and to determine the degree of variation in yields between two sets of identical ammonia and water load parameters at a temperature difference of 60 degrees from each other. It should be noted that there is no record in this first set of the water soaking time prior to AFEX treatment. The soak time is a valuable piece of information about the water equilibration and the net ammonia diffusion rate result. there would be some real confusion over the interpretation, since both 30°C wet and 90°C dry at very similar ammonia loading (1:1/90°C and 1.5:1/30, °C with respect to the pressure and diffusivity enhancement alone) look very much alike in resulting glucan digestibility of just over 30% . The actuality of this first set is that there are far too many variables and the interpretation cannot be very conclusive based on just the facts presented in data Set 1. This is exactly the reason that several data sets were required to begin to conclude any correlation between the data set parameter and the % Glucan Digestibility.

### ***Reaction Time While Soaking in Ammonia is Made Uniform in All Observations***

The decision to delete the water soaking time and hold all observations constant with respect to the length of time ammonia is exposed to the biomass is made when formulating the parameters for data Set 2. These observations use a predetermined length of time for each observation which is greater than the ramp time for any temperature plus thirty minutes. Records were kept on the first set of data which revealed the ramp time necessary to achieve each observation temperature. A value of 55 minutes was decided upon and used in Set 2 except for the very first observation which was over shot by about eight minutes.

**Table 10. AFEX Extended Time (Water held constant) 2X Vs 3X NH3 Observations**

**Enzyme Saccharification of Hybrid Poplar Test Samples (NREL Protocol #009):**

**SET # 2** ( Particle Size: - 60 mesh)-NREL knife milled and sieved to particle size specified.

**AFEX Treatment Conditions:**

**%DIGESTIBILITY:**

	<u>72hrs</u>
01- Untreated hybrid poplar	15.0%
02- 90° C, 3:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(63 min.)	49.0 %
03- 90° C, 2:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55 min.)	42.0 %
04- 70° C, 3:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55 min.)	42.3 %
05- 70° C, 2:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55 min.)	48.2 %
06- 50° C, 3:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55 min.)	37.5 %
07- 50° C, 2:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55 min.)	37.1 %
α Cellulose Standard	87.0%

(Sigma brand)- is this alpha cellulose value which belongs with this set of data

***Substrate Composition and Sieving / Milling Techniques Explain Variations in Results***

Observations 2 at 49% and 5 at 48.2 % in Set 2 are extremely close in glucan digestibility. These 90° and 70°C observations vary by only eight tenths of a percent and Observation 2 at 90°C was really run for eight minutes longer than Observation 5 at 70°C. It appears that similar results can be achieved at 20°C lower and 1Kilogram of ammonia less .per kilogram of dry hybrid poplar through Observation 5 as compared to Observation 2. This is a very significant finding which will be utilized in the energy and mass balance and later will greatly influence the commercial cost of this process per kilogram of dry hybrid poplar. Another example of the 70°C effectiveness is revealed in Observation 4 of Set 2 which probably runs slightly lower due only to the excess ammonia and less aggressive explosion generated at the lower reactor temperature. Observations 4, 5 and 6 of data Set 4 are run at 70°C and yield 47.25, 46.94 and 46.94% glucan digestibility respectively. There seems to be no significant improvement in Observation 5 when 1kg/kg dry biomass more ammonia is utilized.

Observations made in data Set 3 include Observation 1 on page 17, which is treated with all parameters identical to , Observation 4, of data Set 2 page 15 have comparative yields only after 168 hours in set 3 as compared to 72 hours in Set 2 data. Data Set 2 is listed as a 72 hour run but Observation 4 at 72 hours is reading considerably higher than Observation 1 in data Set 3 even after 168 hours. The untreated hybrid poplar and the alpha cellulose in Set 2 at the 72 hour reading, are already at the same values they achieve in Set 3 only after 168 hours.

It looks as though Set 2 was run at possibly a higher enzyme loading, higher temperature or sampled later than 72 hours. There is the possibility that the first set was autoclaved and the second set was not or that there was enough difference in the poplar substrate sampling to account for these differences. Basically the trend can be established by relying upon the alpha cellulose and untreated wood values to standardize the observations. Since the alpha cellulose and the untreated wood values in Set 2 (at 72 hours) and Set 3 (at 168 hours) are extremely close we are assuming the biomass should be comparable by using this relationship.

Table 11. AFEX Optimized Temperature Wet Vs Dry 1X Vs 2X NH3 Observations

Enzyme Saccharification of Hybrid Poplar Test Samples (NREL Protocol #009):

**SET #3** (Particle Size: -60 Mesh)-NREL knife milled and sieved to particle size specified.

AFEX Treatment Conditions:		% Digestibility :	
		72hrs	168hrs
01-	70° C, 3:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	31.01%	36.27%
02-	85° C, 2:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	35.69 %	41.00 %
03-	70° C, 3:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	29.43 %	33.44 %
04-	70° C, 2:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	25.61 %	31.14 %
05-	70° C, 3:1 NH <sub>3</sub> , dry (85min)	29.88 %	35.64 %
06-	70° C, 2:1 NH <sub>3</sub> , dry (85min)	28.08 %	33.44 %
07-	85° C, 3:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	30.2 %	32.27 %
08-	Untreated hybrid poplar	12.92 %	15.08 %
	α Cellulose Standard (NREL)	76.91 %	86.24 %

Table 12. AFEX Optimized Temperatures Larger Particles Wet Vs Dry Observations

Enzyme Saccharification of Hybrid Poplar Test Samples (NREL Protocol #009):

**SET # 4** ( Particle Size: 1/16 in. + 60 mesh)-NREL knife milled and sieved to particle size specified.

AFEX Treatment Conditions:		%DIGESTIBILITY:	
		72hrs	168 hr.
01-	85° C, 3:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood (55min)	45.45 %	52.02 %
02-	85° C, 2:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	39.78 %	44.51%
03-	70° C, 3:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	39.69 %	43.20 %
04	70° C, 2:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	41.81 %	47.25 %
05	70° C, 3:1 NH <sub>3</sub> , 0.0 kg H <sub>2</sub> O per kg wood(85min)	42.84 %	46.94 %
06	70° C, 2:1 NH <sub>3</sub> , 0.0 kg H <sub>2</sub> O per kg wood(85min)	42.80 %	46.94 %
08	Untreated Hybrid Poplar	11.16 %	12.56 %
	α Cellulose Standard (NREL)	74.52 %	86.13 %

**Table 13. AFEX Optimized Temperature, Reduced Ammonia, Wet Vs Dry & Time Increase**

**Enzyme Saccharification of Hybrid Poplar Test Samples (NREL Protocol #009):**

**SET # 5 ( Particle Size: - 60 mesh)-MBI Double disk attrition milled by "Winona" mill.**

AFEX Treatment Conditions:		%DIGESTIBILITY: <u>72hrs</u>
01-	85° C, 1.5:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	31.41 %
02-	85° C, 1:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	29.39 %
03-	70° C, 1:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	24.93 %
04	85° C, 1:1 NH <sub>3</sub> , dry (85min) wood	22.14 %
05	70° C, 1:1 NH <sub>3</sub> , dry (85min) wood	22.05 %
06	Untreated Hybrid Poplar	13.91 %
	α Cellulose Standard (NREL)	71.46 %

**Table 14. AFEX Observations on MBI Milled Poplar to Reproduce NREL Poplar Results**

**Enzyme Saccharification of MBI Milled Poplar Test Samples (NREL Protocol #009):**

**SET # 6 ( Particle Size: -40 mesh) MBI Double disk attrition milled by "Winona" mill.**

AFEX Treatment Conditions:		%DIGESTIBILITY:	
		<u>72 hr.</u>	<u>168 hr.</u>
01-	85° C, 3:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O/kg wood(55min)	30.24 % (30.56)	35.91 % (36.23)
02-	85° C, 2:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O/kg wood(55min)	27.68 % (21.33)	38.97 % (32.63)
03-	70° C, 3:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O/kg wood(55min)	29.48 % (23.40)	38.21 % (32.13)
04	70° C, 2:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O/kg wood(55min)	19.22 % (16.92)	23.18 % (20.88)
05	70° C, 3:1 NH <sub>3</sub> , dry wood(85min)	30.91%(30.15)	34.02% (30.51)
06	70° C, 2:1 NH <sub>3</sub> , dry wood(85min)	28.67 % (29.79)	30.51 % (33.12)
07	Untreated Hybrid Poplar wood	7.65 % (8.46)	8.46 % (9.9)
<b>Control α Cellulose Standard (NREL)</b>		<b>67.37 % (53.82)</b>	<b>78.17 % (70.92)</b>

Table 15. AFEX Observations on Milled MBI Poplar

Enzyme Saccharification of MBI Milled Poplar Test Samples (NREL Protocol #009):  
SET # 7 ( Particle Size: - 40 mesh) MBI Double disk attrition milled by "Winona" mill.

AFEX Treatment Conditions:	%DIGESTIBILITY:	
	<u>72 hr.</u>	<u>168 hr.</u>
01-85° C, 2:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	30.24 %(30.56)	35.91 % (36.23)
02-85° C, 2:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	27.68 %(21.33)	38.97 % (32.63)
03-70° C, 3:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	29.48 %(23.40)	38.21 % (32.13)
04-70° C, 2:1 NH <sub>3</sub> , 0.5 kg H <sub>2</sub> O per kg wood(55min)	19.22 % (16.92)	23.18 % (20.88)
05-70° C, 2:1 NH <sub>3</sub> , dry (85min)	28.67 %(29.79)	30.51 % (33.12)
06-70° C, 2:1 NH <sub>3</sub> , dry (85min)	30.92 % (34. 37)	34.02 % (37.58)
07 Untreated Hybrid Poplar	7.65 % (8.46 )	8.46% (9.9%)

Control      α Cellulose Standard (NREL) .....

Values in parenthesis are obtained using  
the new enzyme.



## Summary

### ***Substrate Composition and Sieving and Milling Techniques Explain Variations in Data***

The subcontract overall goal of optimizing glucan digestibility seems to have been approached best in data Set 4 which utilizes 1/16 inch to + 60 mesh particle size. It is a disappointment that the final hybrid poplar substrate which was shipped to Michigan Biotechnology Institute for milling had a significantly lower (almost 10% lower) digestible glucan content as tested by the glucan digestibility protocol. The type of milling and sieving done by NREL Vs. the milling done at MBI may have had a negative comparison effect as well as possibly creating many variations including undetermined compositional factors that can evolve from particle size sieving alone. The lignin (soluble and insoluble) as well as the ash content appear to fall into the same ranges as the test samples and the substituted substrate. There is the fact that the moisture content is at less than 2% in this final hybrid poplar which suggests heat was generated during milling at MBI as well as possible compacting and compression which would result from the kind of pressure that would be necessary to generate this level of heat. It is known that the hybrid poplar was milled at MBI on an extremely rainy and high humidity day which should not have had an effect of reduced moisture. The reduced moisture fact alone has a significant bearing on the AFEX process and I would assume the acid hydrolysis results as well. Although the results seem inconclusive as compared to the first three sets of data, quite a bit of information can be gleaned from studying these hybrid poplar results for future application of the AFEX process. Overall reproducibility between the two significantly different batches of hybrid poplar was poor. The conclusion is that all biomass processing factors from the field to the end product in an ethanol or bio-fuel production process are critical and have intense economic impact on the final pretreatment effectiveness and commercial feasibility.

### **REVIEW AND COMMENTARY ON ETHANOL EXPERIMENTAL DATA SETS**

Below both the alpha cellulose SSF (Simultaneous Saccharification and Fermentation) which met QA/QC specifications and the fermentation results from some optimized AFEX treatment samples are presented below. Appendix C contains more detail on the fermentation experimental data on Set 1. Repeated digestibility experiments on hybrid poplar samples treated at 90°C; 3:1 ammonia weight to biomass weight ratio and 70°C; 2:1 ammonia/biomass ratio provided comparable results as indicated, e.g., by the most recent experimental results on these samples: 168 hours %digestibility as 45.37 and 45.39 respectively. Although we did not ferment the 70°C sample, the fermentation of this sample should correlate similarly to the digestibility. The trend in the resulting observations was a direct correlation between hybrid poplar enzymatic hydrolysis, glucose yields and ethanol yields using the NREL SSF protocols on AFEX treated and untreated samples.

Table 16. SSF Fermentation Results from NREL Procedure LAP 009

**ETHANOL PRODUCTION VIA FERMENTATION**

**NREL a-Cellulose SSF Data:**

**Optimized AFEX Treated Sample SSF Data:**

AFEX Conditions: 90°C;  
3:1 NH<sub>3</sub>/kg H. Poplar;  
0.5 kg H<sub>2</sub>O/kg H. Poplar;  
-60 mesh Particle size  
56 min. Residence time

% Digestibility (Procedure #009)

72 hours	36.81 %
168 hours	45.39 %

Hydrolysis Under SSF Conditions:

<u>Time(hr.)</u>	<u>Glucose * (mg/dl)</u>	<u>% Yield</u>
0	40	3.6
1	69.5	6.26
3	125.5	11.30
6	138	12.42
12	164	14.76
24	241.5	21.74

\* YSI only

<sup>b</sup> Cellobiose not included

Daily Ethanol Production Rate:

<u>Time (hrs)</u>	<u>Ethanol (g/l)</u>	<u>% Yield (% of the theoretical)</u>
0	2.08 (baseline)	0.0
24	3.35	7.47
48	5.56	20.42
72	7.18	29.91
96	8.58	38.13
120	9.59	44.06
144	10.04	46.72
168	10.24	47.88

Final pH = 5.3

YPD Plate = Only yeast cells were observed

## Appendix A

**MATERIAL AND ENERGY BALANCES IN THE PRETREATMENT OF  
HYBRID POPLAR WOOD  
USING THE AMMONIA FIBER EXPLOSION PROCESS (AFEX)**

In the AFEX process, biomass is subjected to high pressure liquid ammonia at low temperatures. At the end of the required residence time, the pressure is released instantaneously allowing the ammonia to flash and break apart the biomass. The treatment conditions (e.g., temperature-pressure, ammonia/biomass ratio, moisture content, particle size and treatment time) are dependent on the substrate used. For the present study, the following treatment conditions (Table 1.) are considered to be the optimum for hybrid poplar wood. When the treatment temperature is raised to 90°C, compared to the optimum 70°C, the hydrolysis yields did not change significantly and repeated experiments provided almost the same yields at these two temperatures (see page16, Table 10., Set#2, Observations #4 & 5 and page17, Table 12. Set#4, Observations #4,5 & 6). Therefore, to avoid the increased energy cost required for higher temperatures we have chosen the 70°C as the treatment temperature for energy balance calculations.

Biomass: Hybrid poplar wood Particle Size: -40 mesh Solids: 97.3 % Moisture: 2.7 %
---

**Table 1. : AFEX Processing Operating Conditions**

Treatment Pressure	21.5 atm
Treatment Temperature	70°C
Blowdown Pressure	1.0 atm
Treatment Time (Ramp + Reaction)	60 minutes
Water Loading(kg/kg biomass)	0.5
Ammonia Loading (kg/kg biomass)	2.0

The steps of the process are (see page A4, Fig. 2A for the flow chart):

1. Loading of the biomass in the pretreatment tank (unit 1);
2. Loading of water;
3. Introduction of high pressure and high temperature ammonia: At this step steam

is used to heat the pretreatment tank and to keep the contents of the tank at the desired temperature and pressure. This steam will be injected to the tank jacket and has nothing to do with AFEX treatment itself. In the laboratory AFEX unit at Texas A&M university, a heating tape is used for these puposes. A steam jacket would be more appropriate for large scale treatments.

4. Flashing of the contents of the treatment tank into a flash tank at the end of the required treatment time (unit 2, to atmospheric pressure);

5. Recovery of evaporated ammonia from flash tank - about 65 % of the ammonia loaded is flashed from the tank as determined experimentally: Ammonia leaving the flash tank is compressed to 8.5 atm and leaves the compressor as superheated vapor at 155.4°C. This ammonia can either be condensed (condenser 2) or can be fed directly to the pretreatment tank and condense on the biomass, providing 102.8 kcal energy credit. In our calculations we used the latter option;

6. Ammonia recovery from treated biomass by filter pressing and washing (unit 3): This step involves washing off the ammonia with 80°C water from the biomass and filtering, leaving almost negligible amounts of ammonia in the biomass (based on the experimental work);

7. Ammonia recovery from the wash water by steam distillation (unit 4): 99 % of the ammonia in the wash water is recovered through this step. Distillation requires high pressures (10 atm) in order to avoid refrigeration at the top of the column. Energy recovered through the column condenser (1) is lost to the cooling water, however, the energy carried by the bottoms is used in other units of the process;

8. Recycling of ammonia to the pretreatment tank: Almost all of the ammonia fed to the pretreatment tank can be recovered and recycled, requiring very small amounts of make up ammonia for the process (see page A3, Fig. 1A for material and energy balances)

Based on the material and energy balance calculations at the optimum treatment conditions (see Appendix B, also page A3, Fig. 1A for material and energy balances) the cost of pretreating hybrid poplar wood using the AFEX process would be:

291.2 kcal/kg

338.4 kW/metric ton

\*Based on the natural gas prices for industry (Energy Prices and Taxes, Second Quarter 1994, International Energy Agency, p.280), e.g.,

Average price of natural gas/ $10^7$  kilocalories GCV for industry = \$117.95

Cost of AFEX treatment  $\Rightarrow$  \$ 117.95/ $10^7$ /kcal  $\times$  291.2 kcal/kg  $\times$   $10^3$  kg/metric ton = \$3.43 /metric ton.

# MATERIAL AND ENERGY BALANCES

Basis: 1kg of Hybrid Poplar Wood

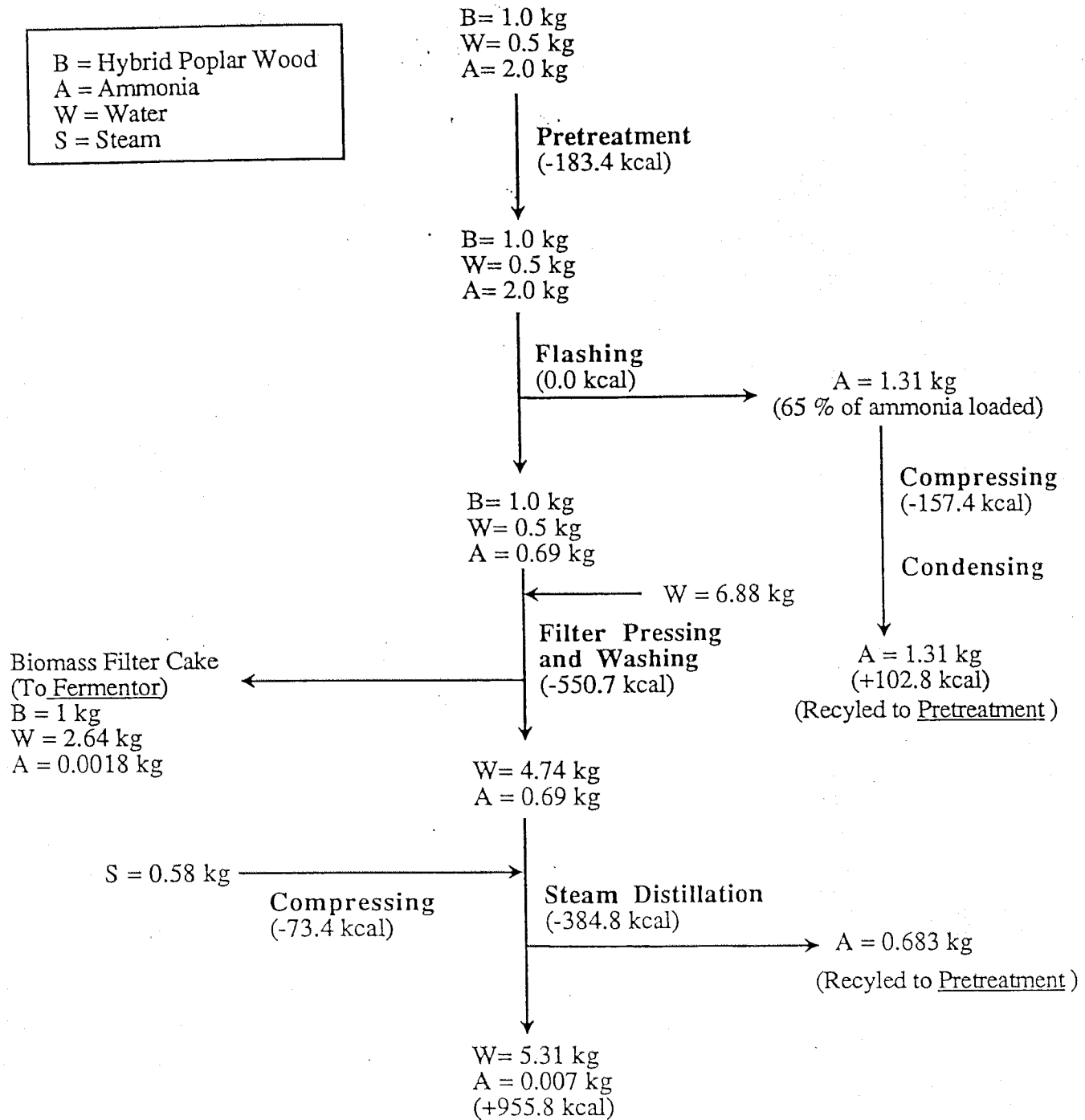


FIGURE 1A

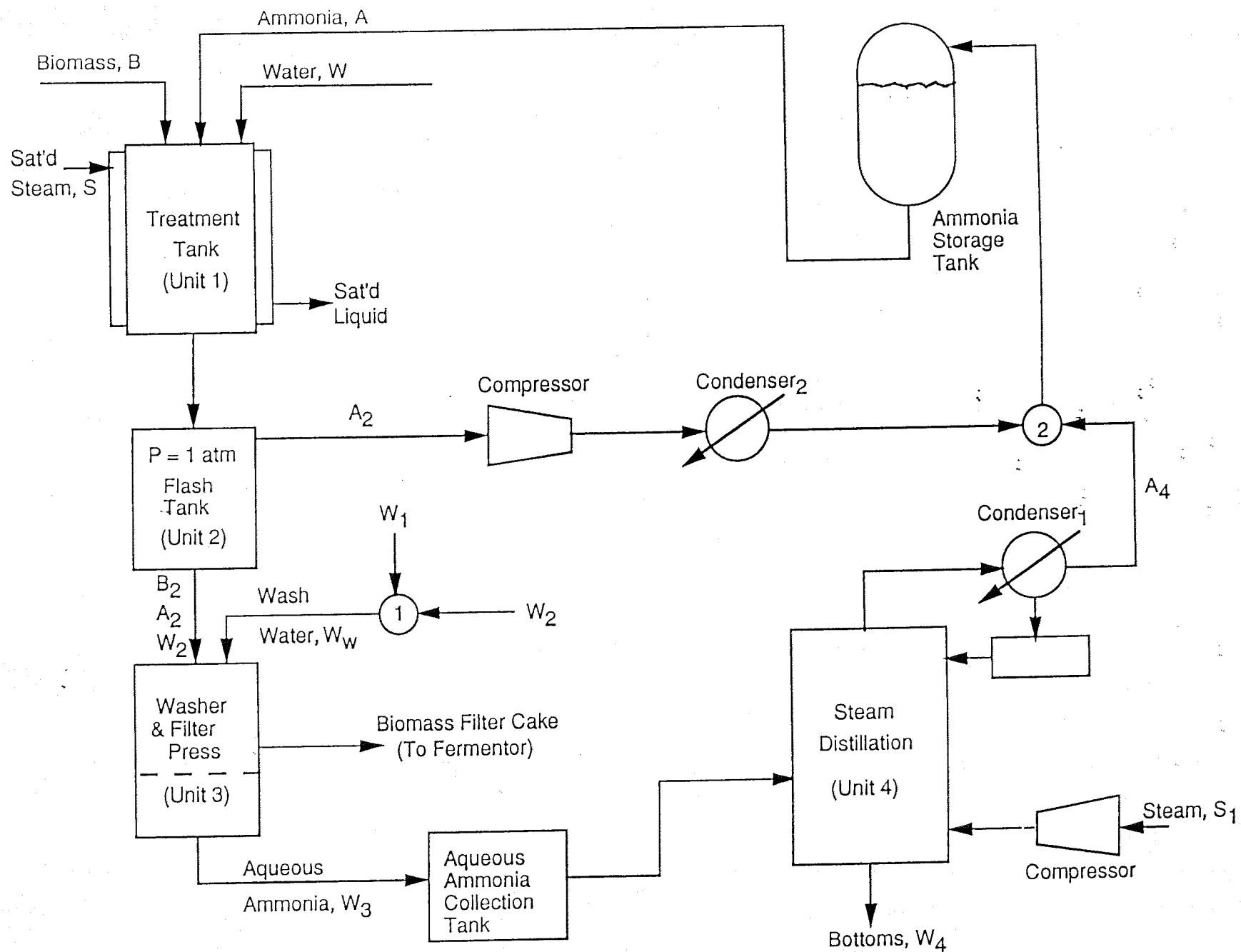


FIGURE 2A - AFEX PROCESS FLOW CHART

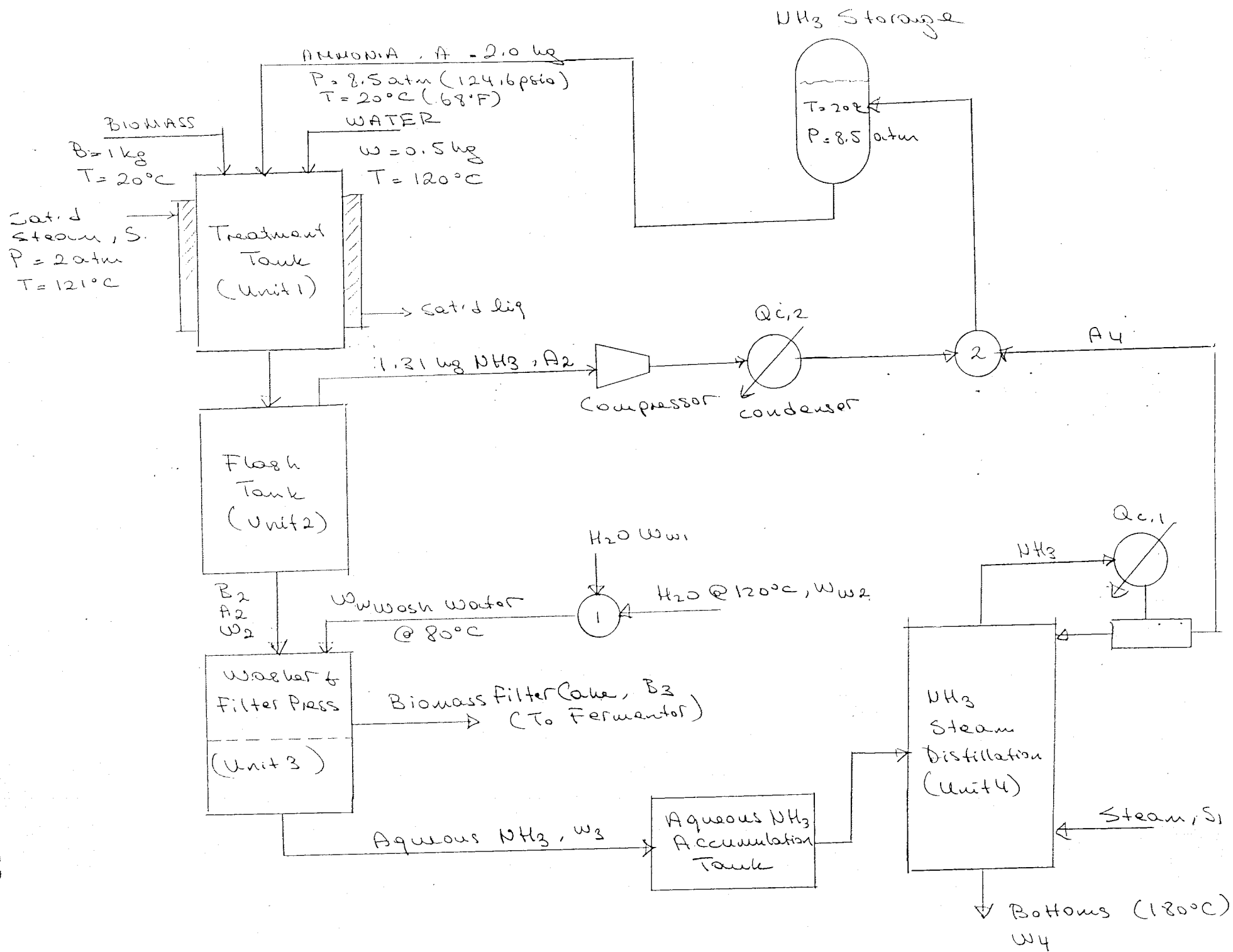
## Appendix B



## Energy Cost:

291.2 kwh / kg biomass

338.4 kw / metric ton of biomass



Biomass: Hybrid Poplar

Particle Size: -40 mesh

Solids: 97.3 %

Moisture: 2.7 %

### AFEX Processing Operation Conditions:

Treatment Pressure: 315.5 psia (21.5 atm)

Treatment Temperature: 70°C (343 K) (158°F)

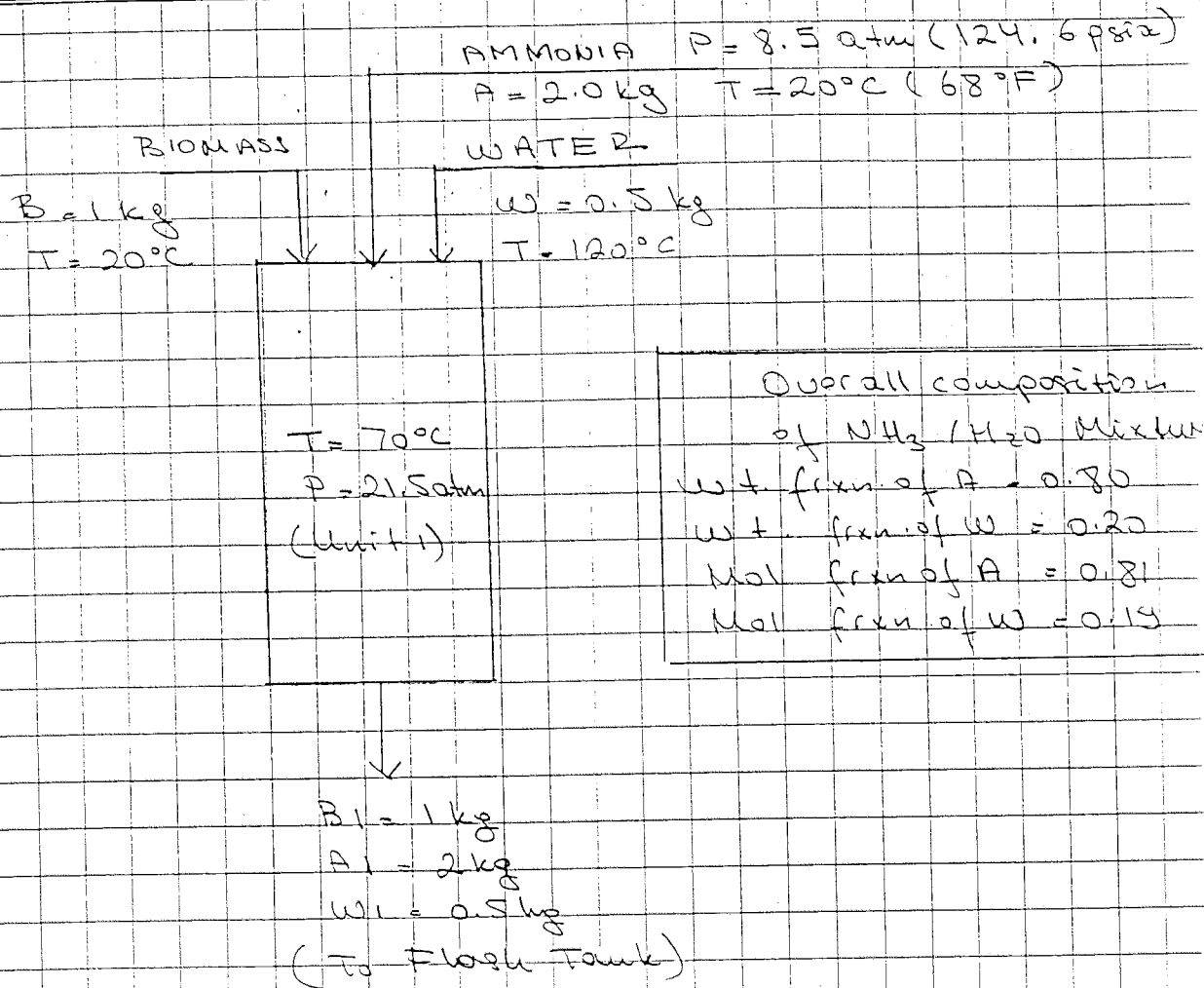
Blowdown Pressure: 1 atm

Treatment Time: 60 min (ramp + rxn)

Water Loading (kg / kg biomass) = 0.5

Ammonia Loading (kg / kg biomass) = 2.0

## TREATMENT TANK (Unit 1)



Basis: 1 kg dry biomass

## $\text{NH}_3/\text{H}_2\text{O}$ Vapor-Liquid Equilibria (Ref 1)

$$T = 340.89 \text{ (67.89}^\circ\text{C)}$$

$$P = 2.175 \text{ MPa (21.5 atm) (315.5 psia)}$$

$$y_A = 0.996 \text{ (mol frxn.)} = 0.996 \text{ (wt. frxn.)}$$

$$x_A = 0.7414 \text{ (mol frxn.)} = 0.7304 \text{ (wt. frxn.)}$$

## Calculation of Vapor/Liquid Ratio in the Treatment Tank

Total Mass Balance:

$$A + W = V_1 + L_1 \quad (1)$$

(3)

Component Balance ( $\text{NH}_3$ ):

$$A = V_1 y_{A,1} + L_1 x_{A,1} \quad (2)$$

$$V_1 + L_1 = 2.5 \implies V_1 = 2.5 - L_1$$

$$2.0 = V_1 (0.936) + L_1 (0.7304)$$

$$2.0 = (2.5 - L_1)(0.936) + L_1 (0.7304)$$

$$2.0 = 2.49 - 0.936 L_1 + 0.7304 L_1$$

$$-0.49 = -0.2656 L_1$$

$$L_1 = \frac{0.49}{0.2656} = 1.845 \text{ kg}$$

$$V_1 = 2.5 - 1.845 = 0.655 \text{ kg}$$

$L_1 = 1.845 \text{ kg}$ $V_1 = 0.655 \text{ kg}$
--

Energy Balance:

$$H_{in} = A \times h_A + W \times h_W$$

$$H_{out} = V_1 h_{V,1} + L_1 h_{L,1} + B \times C_{p,B} (T - T_0) \quad (3)$$

Estimation of thermodynamic properties:

$$h_A (@ 20^\circ\text{C}, \text{sat'd liq}) = 118.24 \frac{\text{Btu}}{\text{lb}} \quad (\text{Reference: liq. NH}_3 @ -40^\circ\text{F} (-40^\circ\text{C}))$$

$118.24 \frac{\text{Btu}}{\text{lbm}}$	$0.239 \times 10^3 \frac{\text{kcal}}{\text{kg}}$	$2.2 \frac{\text{lbm}}{\text{kg}}$	$= 65.54 \frac{\text{kcal}}{\text{kg}}$
	$0.9486 \times 10^3 \frac{\text{Btu}}{\text{kg}}$	$1 \frac{\text{kg}}{\text{kg}}$	

$$h_{A,0} (@ -40^\circ\text{C}, \text{sat. liq}) = 40 \frac{\text{kcal}}{\text{kg}} \quad (\text{Reference: liq. NH}_3 @ -77^\circ\text{C})$$

$$h_A (@ \text{Ref: liq. NH}_3 @ -77^\circ\text{C}) = h_A (@ 20^\circ\text{C}) - h_{\text{ref}} (-40^\circ\text{C}) +$$

$$h_A (-40^\circ\text{C}) - h_{\text{ref}} (-77^\circ\text{C}) =$$

$$h_A (@ 20^\circ\text{C}, \text{sat'd liq}) = 65.54 + 40 = 105.54 \frac{\text{kcal}}{\text{kg}} \quad (\text{D.O.I. liq. NH}_3 @ -77^\circ\text{C} \text{ kg})$$

$$h_{w, \text{ (sat. d. liq. H}_2\text{O @ 120}^\circ\text{C + 2 atm)}} = 120 \text{ kcal/kg} \quad (\text{Ref 2})$$

$$H_{v, \text{ [ @ 20 kg/m}^2 \text{ (284.4 psia), } y_A = 0.996 \text{ ]}} = 398 \text{ kcal/kg (Ref 2)}$$

$$h_{f, \text{ [ @ 20 kg/m}^2 \text{ (284.4 psia), } X_A = 0.7304 \text{ ]}} = 90 \text{ kcal/kg (Ref 2)}$$

$$C_{pB} = 0.55 \text{ kcal/kg}^\circ\text{C}$$

$$H_{in} = 210 \times 105.4 + 0.5 \times 120.0$$

$$H_{in} = 210.8 + 60 = 270.8 \text{ kcal}$$

$$H_{out} = 0.655 \times 398 + 1.845 \times 90 + 1 \times 0.55 (70 - 20)$$

$$H_{out} = 260.63 + 166.05 + 27.5$$

$$H_{out} = 454.24 \text{ kcal}$$

$$H_{out} - H_{in} = Q$$

$$Q = 454.24 - 270.8 = 183.44 \text{ kcal}$$

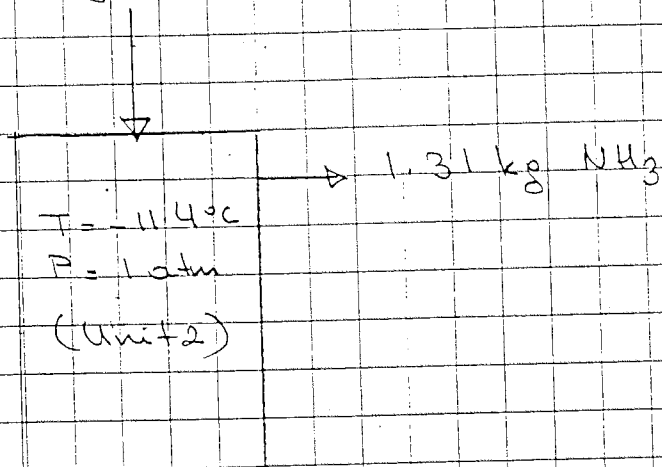
$$Q = 183.44 \text{ kcal}$$

→ Heat required for pretreatment

(5)

Flash Tank (Unit 2)

1 kg biomass  
 2 kg  $\text{NH}_3$   
 0.5 kg  $\text{H}_2\text{O}$



1 kg biomass  
 0.69 kg  $\text{NH}_3$   
 0.5 kg  $\text{H}_2\text{O}$

Energy Balance for isenthalpic flash

$$H_{in} = H_{out}$$

$$H_{in} = V_1 \times H_v(70^\circ\text{C}, 20\text{atm}, y_A = 0.996) + \\ L_1 \times h_L(70^\circ\text{C}, 20\text{atm}, x_A = 0.7304) + \\ m_B \times C_{pB}(T - 0^\circ\text{C})$$

$$H_{out} = V_2 \times H_v(T, 1\text{atm}, y_A) + \\ L_2 \times h_L(T, 1\text{atm}, x_A) + \\ m_B \times C_{pB}(T - 0^\circ\text{C})$$

(6)

Measured temperature of the biomass after the explosion =  $10^{\circ}\text{C} - 7^{\circ}\text{C}$   
 $=$  experimentally determined

From VLE data\* @  $y_{\text{NH}_3} = 0.567$  @ 1 atm

$$y_A = 1.0 \quad (\text{wt \%})$$

$$x_A = 0.567 \quad (\text{wt \%})$$

\* Clifford & Hunter  
1932

$$T = -11.4^{\circ}\text{C}$$

$$\begin{aligned} H_{in} &= 0.655 \times 398 + 1.845 \times 90 + 1 \times 0.55 (70 - 0) \\ &= 260.69 + 166.05 + 38.5 \\ &= 465.24 \text{ kcal/kg dry biomass} \end{aligned}$$

$$H_v (@ 1 \text{ atm}, -11.4^{\circ}\text{C}, y = 1.0) = 375 \text{ kcal/kg (Perry)}$$

$$h_L (@ 1 \text{ atm}, -11.4^{\circ}\text{C}, x = 0.567) = -20 \text{ kcal/kg (Perry)}$$

$$H_{out} = V_2 \times 375 + L_2 \times -20 + 1 \times 0.55 (-11.4 - 0^{\circ}\text{C})$$

$$H_{out} = 375 V_2 - 20 L_2 - 6.27$$

$$465.24 = 375 V_2 - 20 L_2 - 6.27$$

$$471.51 = 375 V_2 - 20 L_2 \quad (1)$$

Mass Balance

$$V_1 \times y_{A,1} + L_1 \times x_{A,1} = V_2 \times y_{A,2} + L_2 \times x_{A,2}$$

$$0.655 \times 0.986 + 1.845 \times 0.7304 = V_2 \times 1.0 + L_2 \times 0.567$$

$$2.0 = V_2 + 0.567 L_2 \quad (2)$$

Solve (1) & (2)

$$471.51 = 375 (2 - 0.567 L_2) - 20 L_2$$

$$471.51 = 750 - 221.13 L_2 - 20 L_2$$

$$471.51 = 750 - 241.13 L_2$$



$$V_2 = 1.345 \text{ kg}$$

$$L_2 + V_2 = 2.564$$

$$L_2 + V_2 \text{ Should be } 2.527$$

From mass balance:

$$L_2 = \underbrace{0.5}_{\text{H}_2\text{O}} + \underbrace{2 \times 0.3456}_{\substack{\text{NH}_3 \text{ left} \\ \text{from NH}_3 \text{ recovery} \\ \text{experiments}}} + \underbrace{1 \times 0.027}_{\text{H}_2\text{O in biomass}}$$

$$L_2 = 1.2182 \text{ kg}$$

$$V_2 = 1.3088 \text{ kg}$$

{ 65.44 % flushed  
34.56 % remains with  
Biomass }

(8)

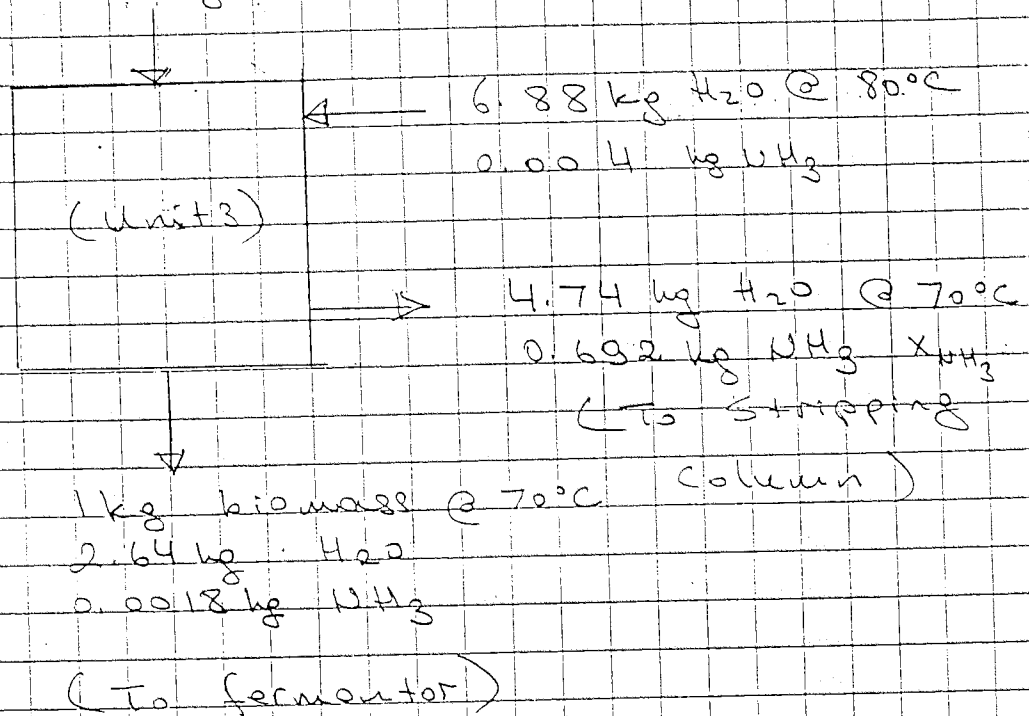
Water & Press (Unit 3)

e.g. A screw press with a filter can be used

1 kg Biomass

0.63 kg  $\text{NH}_3$

0.5 kg  $\text{H}_2\text{O}$  @  $25^\circ\text{C}$  ( $0^\circ\text{C}$ )



Solubility of  $\text{NH}_3$  in  $\text{H}_2\text{O}$  @  $70^\circ\text{C}$  = 13% (wt)

Assume 10% Solubility @  $70^\circ\text{C}$

Amt. of water required to remove 99.7% of the entering  $\text{NH}_3$ .

$$0.63 \times 0.997 / 0.1 = 6.88 \text{ kg } \text{H}_2\text{O} @ 80^\circ\text{C}$$

Water balance:

$$0.5 + 6.88 - 2.64 = 4.74 \text{ kg}$$

$\text{NH}_3$  balance

$$0.63 + 0.004 = 0.634 = 0.0018 + m \text{ NH}_3$$

$$m \text{ NH}_3 \text{ in wash stream} = 0.634 - 0.0018 = 0.632$$

10E

(9)

Energy Balance:

$$\begin{aligned}
 & m_B \cdot C_{PB} (25-0) + m_{NH_3} \cdot C_{P,NH_3} (25-0) + m_w \cdot C_{pw} (25-0) \\
 & + m_{ww} \cdot C_{pw} (80-0) - m_B \cdot C_{PB} (T-0) + \\
 & m_{NH_3} \cdot C_{P,NH_3} (T-0) + (m_w + m_{ww}) \cdot C_{pw} (T-0)
 \end{aligned}$$

$$1.0 \times 0.933 \times 25 + 0.69 \times 1.254 \times 25 + 0.5 \times 1.0 \times 25$$

$$+ 6.884 \times 1.0 \times 80 = (1 \times 0.55 + 0.634 \times 1.254 +$$

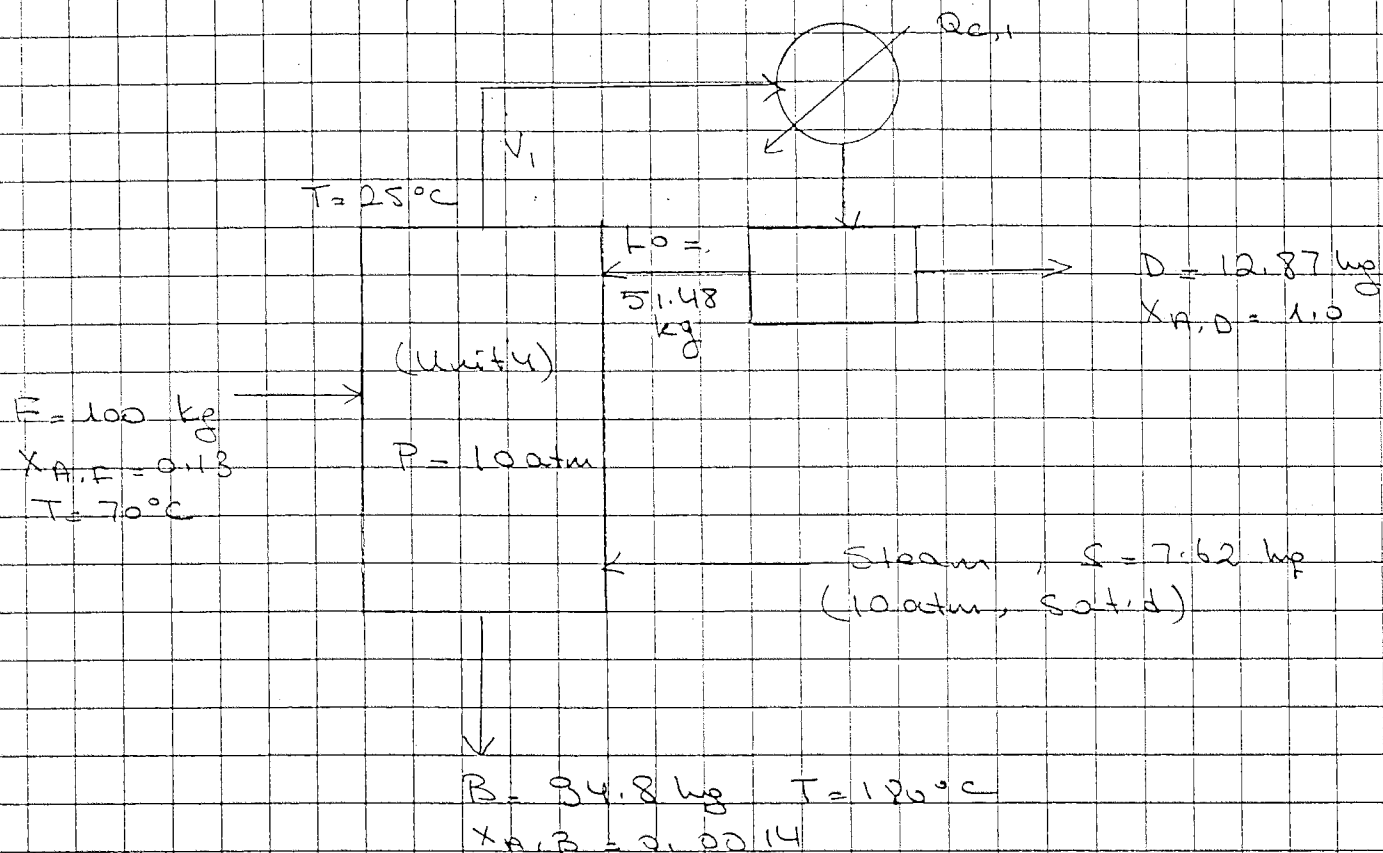
$$7.38 \times 1.0) \times T$$

$$2.325 + 21.6315 + 12.5 + 550.72 = 8.8 T$$

$$T = \frac{587.18}{8.8}$$

$$T = 66.72^\circ C$$

## Steam Distillation of $\text{NH}_3$ : (Unit 4)



Basis: 100 kg feed ( $X_{A,F} = 0.13$ )

Amount of  $\text{NH}_3$  in  $F = 100 \times 0.13 = 13 \text{ kg}$

would like to recover 99% of  $\text{NH}_3$  in feed stream

$A \text{ in } D = 13.0 \times 0.99 = 12.87 \text{ kg}$

$A \text{ in } B = 0.13 \text{ kg}$

$\therefore D = 12.87 \text{ kg} \quad (X_{A,D} = 1.0)$

Overall Mass Balance

$$F + S = D + B$$

$$B = F + S - D$$

$$B = 100 + S - 12.87 \Rightarrow B = 87.13 + S \quad (1)$$

Fix reflux ratio,  $L_0/D = 4.0$

$$L_0 = 4 \times 12.87 = 51.48 \text{ kg}$$

$$V_1 = L_0 + D = 51.48 + 12.87 = 64.35 \text{ kg}$$

Energy Balance for the overall column

$$F \times h_F + S \times H_S = D \times h_D + B \times h_B + Q_c$$

$$h_F (70^\circ\text{C}, X_A = 0.13, P = 10 \text{ atm}) = 130 \text{ kcal/kg (Ref. 2)}$$

$$H_S (\text{sat'd steam @ } 10 \text{ atm}) = 2776.2 \text{ kJ/kg} \times \frac{0.239}{1 \text{ kJ}} = 663.5 \text{ kcal/kg}$$

$$h_D (\text{sat'd liq. } \text{NH}_3 \text{ @ } 10 \text{ atm}) = 129.1 \frac{\text{Btu}}{\text{lb}}$$

$$= 71.6 \text{ kcal/kg}$$

$$= 71.6 + 40$$

$$= 111.57 \text{ kcal/kg}$$

$$h_B (10 \text{ atm}, X_{A,B} \approx 0.0, 180^\circ\text{C}) = 180 \text{ kcal/kg}$$

$$100 \times 130 + S \times 663.5 = 12.87 \times 111.6 + B \times 180 + Q_c$$

Calculation of condenser duty,  $Q_c$ :

$$h_{fg} (\text{@ } 10 \text{ atm}) = h_g - h_f$$

$$h_f = 111.6 \text{ kcal/kg}$$

$$h_g = 630.3 \text{ Btu/lb} = 349.4 \text{ kcal/kg} + 40 = 389.4$$

$$h_{fg} (\text{@ } 10 \text{ atm}) = 389.4 - 111.6 = 277.8 \text{ kcal/kg}$$

$$Q_c = V_1 \times h_{fg} = 64.35 \text{ kg} \times 277.8 = 17,876.4 \text{ kcal}$$

$$13,000 + 663.5 S = 1,436.3 + 180 B + 17,876.4$$

$$663.5 S - 180 B = 6,312.7 \quad (2)$$

Solve (1) + (2) simultaneously for B + S:

$$663.5 S - 180 (87.13 + S) = 9,370$$

$$663.5 S - 15,683.4 - 180 S = 9,370$$

$$583.5 S = 6,313$$

$$S = 10.62 \text{ kg}$$

$$B = 87.13 + S = 87.13 + 10.62$$

$$B = 97.95 \text{ kg}$$

$\text{NH}_3$  balance:

$$F \times X_{\text{NH}_3, F} = D + B \times X_{\text{NH}_3, B}$$

$$13.0 = 12.87 + 94.8 \times X_{\text{NH}_3, B}$$

$$X_{\text{NH}_3, B} = \frac{0.13}{94.8} = 0.0014$$

Calculation of # of stages.

Stripping Section

$$y_m = \frac{\bar{L}}{\bar{V}} x_{m-1} - \frac{\bar{L} x_N - \bar{V} y_{N+1}}{\bar{V}}$$

Assume: Constant molar overflow

$$\bar{L} = B$$

$$\bar{V} = S$$

$$y_m = \frac{B}{S} x_{m-1} - \frac{B \cdot x_{A,B} - S \cdot y_{A,S}}{S} \quad (3)$$

Operating line for the stripping section

Enriching section:

$$y_{n+1} = \frac{L_0}{V_1} x_n + \frac{D}{V_1} x_{A,D} \quad (4)$$

$$L = L_0$$

$$V = V_1$$

Eqn. (3)

$$y_m = \frac{94.8}{7.62} x_{m-1} - \frac{94.8 \times 0.0014}{7.62}$$

$$y_m = 12.44 x_{m-1} - 0.018 \quad (3)$$

Eqn. (4)

$$y_{n+1} = \frac{51.48}{64.35} x_n + \frac{12.87}{64.35} \times 1.0$$

$$y_{n+1} = 0.8 x_n + 0.2 \quad (4)$$

Intersection

$$12.44 X_A - 0.018 = 0.8 X_A + 0.2$$

$$11.64 X_A = 0.218$$

$$X_A = 0.0186$$

$$y_A = 0.269$$

Eqn. (3)

$$X_A = 0$$

$$y_A = -0.018$$

Eqn. (4)

$$X_A = 1.0$$

$$y_A = 1.0$$

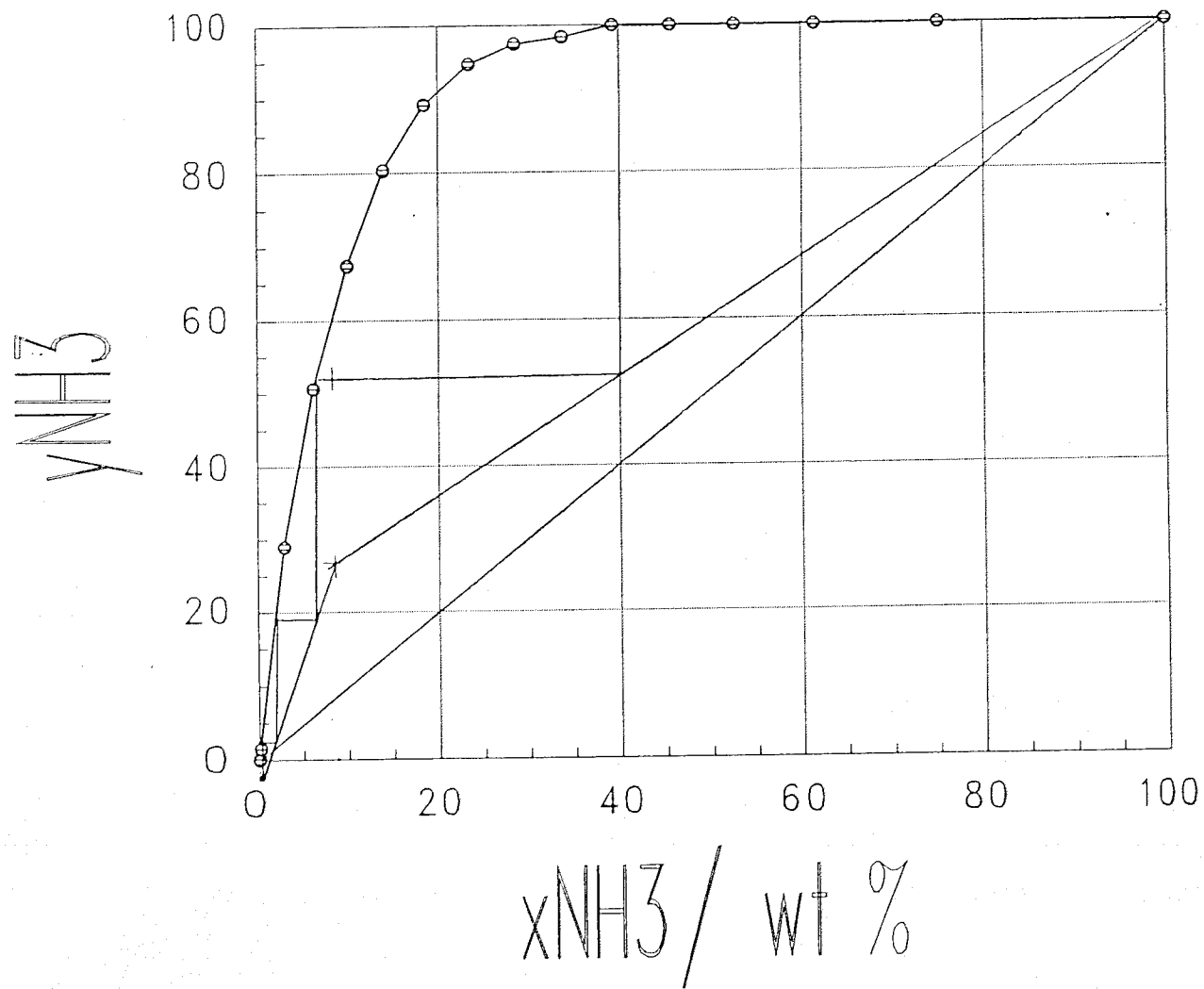
$\therefore$  # of theoretical stages = 4

Scale down for 1 kg biomass basis.

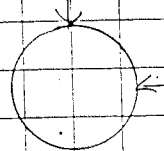
$F = 5.428$ kg / kg biomass
$D = 0.7$ kg
$B = 5.31$ kg
$S = 0.58$ kg
$L_0 = 2.79$

$$Q_{c,1} = \underline{\underline{77.6.3 \text{ kcal}}}$$





(1b)

Mixing Point! #1 $m_{cw} @ 25^\circ C$  $m_{Bw} @ 120^\circ C$  Sat'd. liq. $m_w @ 80^\circ C$ 

$$m_{Bw} \times h_B + m_{cw} \times C_{p,cw} (25 - 0) = (m_{Bw} + m_{cw}) (80 - 0)$$

$$m_{Bw} \times 120 + m_{cw} \times 1 \times 25 = 80 m_{Bw} + 80 m_{cw}$$

$$40 m_{Bw} = 55 m_{cw}$$

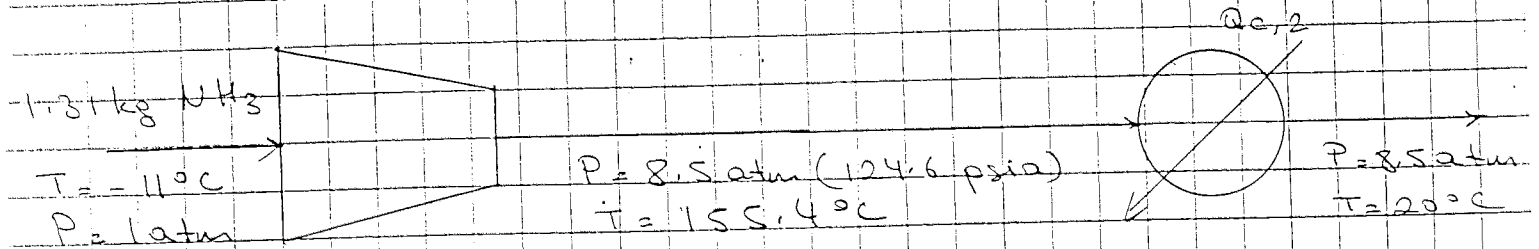
$\frac{m_{cw}}{m_{Bw}} = 0.727$
---------------------------------

$$m_{cw} + m_{Bw} \times 0.999 = 6.884 \text{ — Amt of wash water req'd}$$

$$0.727 m_{Bw} + 0.999 m_{Bw} = 6.88$$

$$m_{Bw} = \frac{6.88}{1.726} = 3.99 \text{ kg}$$

$$(cw) m_{cw} = 2.89 \text{ kg}$$

COMPRESSOR

Adiabatic discharge temperature:

$$T_2 = T_1 \left( \frac{P_2}{P_1} \right)^{\frac{k-1}{k}}$$

$$T_2 = (-11 + 273.2) \left( \frac{124.6}{14.7} \right)^{\frac{1.3-1}{1.3}}$$

$$T_2 = (262.2) \left( \quad \right) = 428.1 \text{ K} \\ = 155.4^\circ \text{C}$$

Compressor energy required:

$$W = \frac{k}{k-1} RT_1 \left[ \left( \frac{P_2}{P_1} \right)^{\frac{k-1}{k}} - 1 \right]$$

$$W = \frac{1.3}{1.3-1} \cdot 1.987 \frac{\text{cal}}{\text{mol} \cdot \text{K}} \cdot \frac{(-11 + 273.2)}{262.2} \left[ \left( \frac{124.6}{14.7} \right)^{\frac{0.3}{1.3}} - 1 \right]$$

$$W = 1,432.9 \frac{\text{kcal}}{\text{kmol}} \times \frac{1 \text{ kmol}}{17.03 \text{ kg}} = 84.14 \frac{\text{kcal}}{\text{kg}}$$

$$\Sigma W = 1.31 \times 84.14 = 110.2 \text{ kcal}$$

Assume: 70% Compressor efficiency

$$\Sigma W = 157.43 \text{ kcal}$$

Condenser duty,  $Q_{c,2}$ :

$$Q_{c,2} = H_{in} - H_{out} =$$

$$H_{in} (149^\circ\text{C}, 8.5 \text{ atm superheated } \text{NH}_3) = 769.3 \frac{\text{Btu}}{\text{lb}}$$

$$H_{out} (20^\circ\text{C}, 8.5 \text{ atm sat'd liquid}) = 118.24 \frac{\text{Btu}}{\text{lb}}$$

$$Q_{c,2} = H_{out} - H_{in} = 118.24 - 769.3 = -651.06 \frac{\text{Btu}}{\text{lb}}$$

$$= -360.88 \frac{\text{kcal}}{\text{kg}}$$

$$Q_{c,2} = (1.31) (-360.88) = -472.8 \text{ kcal}$$

$$H_{out} (\text{sat'd } \text{NH}_3 \text{ vapor @ } 8.5 \text{ atm}) = 628.8 \frac{\text{Btu}}{\text{lb}}$$

125 psia

$$= 348.8 \frac{\text{kcal}}{\text{kg}}$$

$$\Delta H = 140.5 \frac{\text{Btu}}{\text{lb}} = 77.93 \frac{\text{kcal}}{\text{kg}}$$

$$Q = 1.31 \text{ kg} (77.93) = -102.08 \text{ kcal}$$

Compressor energy required for high pressure steam.

$$W = \frac{1.395}{1.395 - 1} \times 1.987 (121 + 273.2) \left[ \left( \frac{147}{29.4} \right)^{\frac{0.395}{1.395}} - 1 \right]$$

$$W = 1,596.1 \frac{\text{kJ}}{\text{kmol}} \times \frac{1 \text{ kmol}}{18.02 \text{ kg}} = 88.6 \frac{\text{kJ}}{\text{kg}}$$

$$\Sigma W = 88.6 \times 0.58 = 51.4$$

Assume 70% compressor efficiency

$$\Sigma W = 73.43 \text{ kJ}$$

SUMMARY :

Basis : 1 kg dry hybrid poplar

Required energies for each unit

Pre-treatment : +183.4 kcal

Distillation unit : +384.8 kcal

Compressor : +157.43 kcal

Filter press + washer = +550.72 kcal

Condenser Duty,  $Q_{c1}$  = - 970.3 kcal (cannetula)

Condenser Duty,  $Q_{c2}$  = -102.8 kcal

High pressure steam (compression) = +73.43 kcal

Energy carried in bottoms = 955.8 kcal

Energy requirement for treatment of 1 kg biomass:

$$384.8 + 183.4 + 157.43 + 550.72 + 73.43 - 102.8 - 955.8 = 291.18 \text{ kcal/kg}$$

Biomass Flowrate : 40 tons/hr

Energy in kW	40 tons/hr	1,000 kg/ton	291.18 kcal/kg	1 kJ
				0.239

$\frac{1 \text{ hr}}{3,600 \text{ s}}$	$= 13,536.9 \text{ kW} / 40 \text{ tons of biomass}$
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## REFERENCES:

- 1- Rizvi, S.S.H. and Heldeman, R.A., Vapor-Liquid Equilibria in Ammonia-Water System, *J. Chem. Eng. Data*, **32**, 183 (1987).
- 2- *Perry's Chemical Engineers' Handbook*, 6th Edition.
- 3- Clifford, I.L. and Hunter, E., The System Ammonia-Water at Temperatures up to 150°C, and at Pressures up to Twenty Atmospheres, *J. Phys. Chem.*, **37**, 101 (1933).

# ①

## NH<sub>3</sub> Recovery for 70°C, 2:1, 50% (60 min)

$w_1$  = wt of the loaded biomass  
(dry biomass + H<sub>2</sub>O + NH<sub>3</sub>)

$w_2$  = wt of the wash water (H<sub>2</sub>O + NH<sub>3</sub>)

$$w_1 = 2.7 \text{ g}$$

$$w_2 = 752.2 \text{ g}$$

Basis = 1 g dry biomass

$$w_1 = m_B + \underbrace{0.5416 \text{ g H}_2\text{O} / \text{g dry biomass}}_{(50 + 2.7) / 97.3} \times m_B +$$

NH<sub>3</sub> in wash water,  $m_1, \text{NH}_3$  +  
NH<sub>3</sub> in biomass,  $m_2, \text{NH}_3$

$$\% \text{NH}_3 \text{ in wash water} = \underbrace{0.0931\%}_{\text{Avg \% N}} / 0.8225 = 0.1131\%$$

$$m_1, \text{NH}_3 = 752.2 \times 0.001131 = 0.851 \text{ g NH}_3$$

$$m_2, \text{NH}_3 \Rightarrow$$

Treated & washed = 2.014 mg N / g dry biomass  
Untreated = 0.752 mg N / g dry biomass

$$(2.014 - 0.752) / 0.8225 = 1.534 \text{ mg NH}_3 / \text{g dry bio}$$

$$w_1 = m_B + 0.5416 \times m_B + 0.851 + 1.534 \times 10^{-3} \times m_B$$

$$2.7 = 0.851 + 1.5416 \times m_B + 0.001534 m_B$$

$$m_B = \frac{1.849}{1.543} = 1.198 \text{ g dry matter}$$

44.28 %

74 R



$$\textcircled{1} \text{ NH}_3 / \text{dry matter} = 200 / 97.3 = 2.06$$

$$\textcircled{2} \text{ NH}_3 / \text{dry matter after explosion} = \frac{0.851 + 0.0018}{1.198}$$

$$= 0.712$$

$$\% \text{ flashed} = 65.44$$

$$\% \text{ with biomass} = 34.56$$

$$X_{\text{NH}_3} = \frac{200 \times 0.3456}{69.12 + 52.7} = 0.567$$

$$\frac{0.712}{0.712 + 0.5415} = 0.567$$

$$y_{\text{NH}_3} = 1.0 \text{ (Perry)}$$

% NH<sub>3</sub> removed in 1<sup>st</sup> wash:

$$19.3 \text{ gm} \times 0.0036 = 0.6948 \text{ gm} / 1.198$$

$$= 0.58 \text{ gm} / \text{gm dry matter}$$

$$\% \text{ NH}_3 \text{ in the afoxed biomass} = 0.712 \text{ gm} / \text{gm}$$

$$\% \text{ NH}_3 \text{ removed in 1<sup>st</sup> wash} = 0.58 / 0.712$$

$$= 81.46 \%$$

Solid Blank ml 0.0500  
Liquid Blank ml 0.0500  
Acid Normality 0.0537  
Constant 1.4000

TREATMENT	Sample ID	Sample wt (g)	ml of titrant used	% N in sample	Total N (g)	g N/g dry sample
70 °C; 2:1 NH <sub>3</sub> ; 50% H <sub>2</sub> O (60min) After 4 baths	TA after	0.1476	0.2000	0.0764	0.000113	0.002014
70 °C; 2:1 NH <sub>3</sub> ; 50% H <sub>2</sub> O (60min) After 4 baths	TB after	0.1476	0.2000	0.0764	0.000113	0.002014
Untreated	UA	0.0514	0.1000	0.0731	0.000038	0.000752
Untreated	UB	0.0514	0.1000	0.0731	0.000038	0.000752
Total recovered liquid	LTA	2.0000	2.6000	0.0959	0.001917	
Total recovered liquid	LTB	2.0000	2.4500	0.0902	0.001804	
a-Cellulose blank	BaC	0.0052	0.0500			
Distilled water blank	BdW	2.0000	0.0500			
70 °C; 2:1 NH <sub>3</sub> ; 50% H <sub>2</sub> O (60min) Before baths	TA before	0.1476	2.44	1.2173	0.001797	
70 °C; 2:1 NH <sub>3</sub> ; 50% H <sub>2</sub> O (60min) Before baths	TB before	0.1476	2.65	1.3243	0.001955	
Recovered liquid (Bath #1)	L1A	2.0000	9.65	0.3609	0.007217	
Recovered liquid (Bath #1)	L1B	2.0000	9.6	0.3590	0.007180	
Recovered liquid (Bath #2)	L2A	2.0000	0.12	0.0026	0.000053	
Recovered liquid (Bath #2)	L2B	2.0000	0.14	0.0034	0.000068	
Recovered liquid (Bath #3)	L3A	2.0000	0.05	0.0000	0.000000	
Recovered liquid (Bath #3)	L3B	2.0000	0.05	0.0000	0.000000	
Recovered liquid (Bath #4)	L4A	2.0000	0.05	0.0000	0.000000	
Recovered liquid (Bath #4)	L4B	2.0000	0.05	0.0000	0.000000	

Amount of treated biomass captured in the jar = 2.7 g

Volumes of wash liq.

① 193 gm

② 195.5 gm

③ Total volume after 4 washes = 752.2 gm

Moisture content of biomass

after filtration = 0.6208 ∴ Solids = 0.3792

NH<sub>3</sub> Recovery with room temp. water.

$$\begin{aligned} \Rightarrow \text{NH}_3 \text{ in 1st wash} &= 190.9 \times 0.00322 \\ &= 0.615 \text{ gm} / \underbrace{2.4409}_{5.5 \times 0.4438} = 0.252 \text{ gm/g} \end{aligned}$$

$$\Rightarrow \text{NH}_3 \text{ in the dried biomass} = 0.712 \text{ gm/gm}$$

$$\% \text{ NH}_3 \text{ removed with 1st wash} = 35.39 \%$$

Cold Baths ammonia recovery

Liquid Blank ml 0.0500  
 Acid Normality 0.0537  
 Constant 1.4000

TREATMENT	Sample ID	Sample wt (g)	ml of titrant used	% N in sample	Total N (g)	g N/g dry sample
Iowa 70oC ; 2:1 NH3; 50% H2O; 60 MIN (1st w.)	LA1	3.0000	12.9000	0.3220 ✓	0.009661 ✓	0.172511
Iowa 60oC ; 1:1 NH3; 50% H2O; 55 MIN (1st w.)	LB1	3.0000	1.8200	0.0444	0.001331	0.023762
Iowa 70oC ; 2:1 NH3; 50% H2O; 60 MIN (2nd w.)	LA2	3.0000	0.3700	0.0080 ✓	0.000241 ✓	0.004812
Iowa 60oC ; 1:1 NH3; 50% H2O; 55 MIN (2nd w.)	LB2	3.0000	0.1000	0.0013	0.000038	0.000752
Iowa 70oC ; 2:1 NH3; 50% H2O; 60 MIN (3th w.)	LA3	3.0000	0.1000	0.0013 ✓	0.000038 ✓	
Iowa 60oC ; 1:1 NH3; 50% H2O; 55 MIN (3th w.)	LB3	3.0000	0.1000	0.0013	0.000038	
Iowa 70oC ; 2:1 NH3; 50% H2O; 60 MIN (Tot. w.)	LAT	3.0000	4.2000	0.1040	0.003120	
Iowa 60oC ; 1:1 NH3; 50% H2O; 55 MIN (Tot. w.)	LBT	3.0000	0.5800	0.0133	0.000398	
Distilled water blank	Blank	3.0000	0.0500			

2:1, 70°C, Amt. captured in the jar = 5.5 g

1st wash = 190.9 g

2nd = 193.2

3rd = 194.6

Σ Volume after 4th wash = 774.3 g

# NH<sub>3</sub> Recovery for 60°C, 1:1, 50%

(1)

$w_1$  = wt. of the treated biomass

(dry biomass + H<sub>2</sub>O + NH<sub>3</sub>)

$w_2$  = wt. of the wash water (H<sub>2</sub>O + NH<sub>3</sub>)

$$w_1 = 10.6 \text{ g}$$

$$w_2 = 772.6 \text{ g}$$

Basis = 1 g dry biomass

$$w_1 = m_B + \underbrace{0.5416 \text{ g H}_2\text{O/g dry biomass}}_{(50+2.7)/97.3} \times m_B + \text{NH}_3 \text{ in}$$

wash water  $m_{1,\text{NH}_3}$  + NH<sub>3</sub> left in biomass  $m_{2,\text{NH}_3}$

$$m_{1,\text{NH}_3} =$$

$$0.1052\% / 0.8225 = 0.1279\% \text{ NH}_3$$

$$\sum m_{\text{NH}_3} = 772.6 \times 0.001279 = 0.988 \text{ g NH}_3$$

$$m_{2,\text{NH}_3} =$$

$$\text{Treated + washed} = 0.2878 \text{ mg N / g dry}$$

$$(\text{untreated}) = 0.036 \text{ mg N / g dry}$$

$$(0.2159 - 0.036) / 0.8225 =$$

$$0.1799 / 0.8225 = 0.2187 \text{ mg NH}_3 / \text{g dry}$$

$$w_1 = m_B + 0.5416 \times m_B + 0.988 + 0.2187 \times 10^{-3} \times m_B$$

$$10.6 = 1.5416 m_B + 0.988 + 0.0002187 m_B$$

$$m_B = \frac{9.612}{1.5418} = 6.234 \text{ g dry matter}$$

(2)

$$\textcircled{1} \left( \text{NH}_3 / \text{dry Ratio} \right)_{\text{before}} = 100 / 97.3 = 1.028$$

$$\textcircled{2} \left( \text{NH}_3 / \text{dry Ratio} \right)_{\text{after explosion}} = 0.989 / 5.808 = 0.170$$

% 83.43 flashed

% 16.57 remained in biomass

Washed Biomass = % Solids 33.87%

Solid Blank ml	0.1000
Liquid Blank ml	0.0500
Acid Normality	0.0514
Constant	1.4000

TREATMENT	Sample ID	Sample wt (g)	ml of titrant used	% N in sample	Total N (g)	g N/g dry sample
60 °C; 1:1 NH <sub>3</sub> ; 50% H <sub>2</sub> O (55min)	T1	0.1476	0.3000	0.0975	0.000144	0.002878
60 °C; 1:1 NH <sub>3</sub> ; 50% H <sub>2</sub> O (55min)	T2	0.1476	0.2000	0.0488	0.000072	0.001439
Untreated	U1	0.0514	0.1500	0.0700	0.000036	0.000720
Untreated	U2	0.0514	0.1000	0.0000	0.000000	0.000000
Recovered liquid	L1	2.0000	2.9500	0.1043	0.002087	
Recovered liquid	L2	2.0000	3.0000	0.1061	0.002123	
α-Cellulose blank	B1	0.0052	0.1000			
Distilled water blank	B2	2.0000	0.0500			

## Appendix C



# SSF Set#1 Sub-sample A

